



INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(1) International Patent Classification ⁵ : A61K 35/78, 31/365, 31/34, 31/19, 31/12, 31/16, 31/40		A1	(11) International Publication Number: WO 95/00157
			(43) International Publication Date: 5 January 1995 (05.01.95)
(21) International Application Number: PCT/AU94/00342			(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, US, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).
(22) International Filing Date: 24 June 1994 (24.06.94)			
(30) Priority Data: PL 9605 25 June 1993 (25.06.93) AU			
(71) Applicant (for all designated States except US): MOBIUS CONSULTANCY PTY. LTD. [AU/AU]; 2 Anchusa Street, Bellbowrie, QLD 4070 (AU).			
(72) Inventor; and (75) Inventor/Applicant (for US only): DAUNTER, Brian [AU/AU]; 2 Anchusa Street, Bellbowrie, QLD 4070 (AU).			
(74) Agent: KELLY, Robin, Thomas; Fisher & Kelly, Level One, 349 Coronation Drive, Milton, QLD 4064 (AU).			

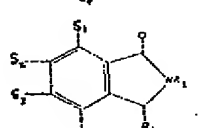
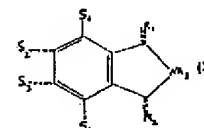
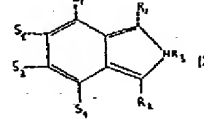
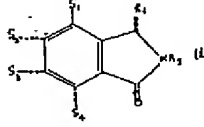
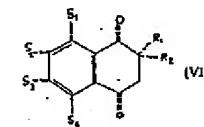
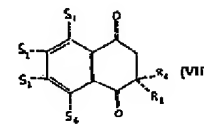
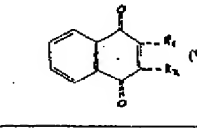
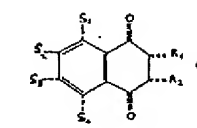
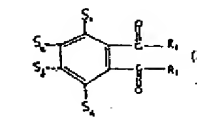
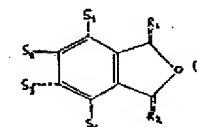
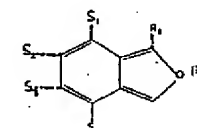
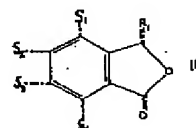
Published

With international search report.

(54) Title: THERAPEUTIC AGENT

(57) Abstract

A method for the treatment of an inflammatory complaint including the step of administration of a compound of general formulae (I to XII) described herein in figure or mixtures thereof to a subject suffering from said inflammatory complaint. There is also included a therapeutic agent for relief of inflammatory complaints which includes a compound of general formulae (I to XII) as described in figure or mixtures thereof in combination with an appropriate filler which is preferably dicalcium phosphate. The invention also includes within its scope plant extracts which may contain one or more of the above-mentioned compounds and in particular plant extracts of the genus *Umbelliferae*, celery (*Apium graveolens*), parsley (*Petroselinum hortense*) and dill (*Anethum graveolens*). It is believed that compounds of the above-mentioned formulae and in particular butylphthalide, sedanenolide and sedanolide (in both cis and trans forms otherwise known as essential oils which are mainly derived from plants) mimic the physiological response of essential fatty acids and also their prostaglandin analogues.



FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AT	Austria	GB	United Kingdom	MR	Mauritania
AU	Australia	GE	Georgia	MW	Malawi
BB	Barbados	GN	Guinea	NE	Niger
BE	Belgium	GR	Greece	NL	Netherlands
BF	Burkina Faso	HU	Hungary	NO	Norway
BG	Bulgaria	IE	Ireland	NZ	New Zealand
BJ	Benin	IT	Italy	PL	Poland
BR	Brazil	JP	Japan	PT	Portugal
BY	Belarus	KE	Kenya	RO	Romania
CA	Canada	KG	Kyrgyzstan	RU	Russian Federation
CF	Central African Republic	KP	Democratic People's Republic of Korea	SD	Sudan
CG	Congo	KR	Republic of Korea	SE	Sweden
CH	Switzerland	KZ	Kazakhstan	SI	Slovenia
CI	Côte d'Ivoire	LI	Liechtenstein	SK	Slovakia
CM	Cameroon	LK	Sri Lanka	SN	Senegal
CN	China	LU	Luxembourg	TD	Chad
CS	Czechoslovakia	LV	Latvia	TG	Togo
CZ	Czech Republic	MC	Monaco	TJ	Tajikistan
DE	Germany	MD	Republic of Moldova	TT	Trinidad and Tobago
DK	Denmark	MG	Madagascar	UA	Ukraine
ES	Spain	ML	Mali	US	United States of America
FI	Finland	MN	Mongolia	UZ	Uzbekistan
FR	France			VN	Viet Nam
GA	Gabon				

TITLE

"THERAPEUTIC AGENT"

FIELD OF THE INVENTION

5 THIS INVENTION relates to a therapeutic agent which may be used for the treatment of inflammatory complaints such as arthritis.

BACKGROUND OF THE INVENTION

10 Arthritis means joint inflammation and really refers to more than 100 rheumatic diseases in which one or more joints are inflamed. Rheumatic illnesses include not only arthritis, but illnesses in which there are aches and pains of the musculoskeletal system, joints, muscles, ligaments, tendons and bursae. Rheumatic syndromes can be classified as inflammatory
15 or degenerative, but this is sometimes less distinct than the nomenclature implies. That is, a joint damaged by inflammatory disease is more vulnerable to degenerative factors, thus syndromes that appear to be degenerative may be associated with inflammation.
20 Hence rheumatic syndromes can virtually present manifestations of numerous types of illnesses. In addition, rheumatic illnesses can be associated with other diseases. This complexity therefore makes diagnosis difficult and its classification tentative
25 (discussed in reference (1) of list of references attached hereto).

The difficulty in diagnosis is demonstratable by the latest consideration given to this complex problem by Webb and Nash in 1990 (2) and similarly this
30 applies to the complexity of classification based on the American Rheumatism Association. It therefore follows that the treatment of rheumatic disease is also complex. This complexity has been addressed by Kremer (3) in 1990, who considers the traditional treatment
35 for rheumatoid arthritis, and proposes modifications to it, and the use of combined chemotherapy and also an experimental approach. These complexities in treatment

also apply to other rheumatic diseases such as osteoarthritis (1).

Arthritis can be associated with infectious agents such as bacteria, fungi and viruses. Thus there is gonococcal arthritis, tuberculous arthritis, syphilitic arthritis, mycotic arthritis and viral arthritis. There is also polyarthritis and viral arthritis. There is also polyarthritis of unknown etiology in which there is prominent involvement of spinal articulations, sacroiliac joints and paravertebral soft tissues. There is neuropathic joint disease which is a progressive degenerative arthropathy classified under miscellaneous forms of arthritis which also includes chondrocalcinosis. Chondrocalcinosis is defined as the presence of calcium containing salts in cartilaginous structures of one or more joints. Because calcium salt crystals are found in joint fluid with attendant chronic synovitis, it has been referred to as pseudogout (1).

Rheumatoid arthritis is a systemic disease of unknown cause, although it does lead to autoimmune expression. The clinical and pathologic findings and resultant disability are the result of chronic inflammation of synovial membranes. The inflamed and hyperplastic synovium contributes to cartilage destruction. It appears that the resident macrophage and fibroblast type cells in the synovium become activated and release mediators of an inflammatory response. In addition, rheumatoid arthritis appears to be exacerbated by an immunological stimulated influx of monocytes/macrophages and leucocytes into the synovial fluid.

There are also variants of the rheumatoid arthritis syndrome (1). In addition, there is the problem of defining or judging disease activity, and this applies to rheumatoid arthritis, the complexity of developing and applying a disease activity score is

exemplified in the latest article by Van der Heijde et al, 1990 (4).

Osteoarthritis, a degenerative joint disease of articular cartilage, probably begins in the second decade of life. It may be considered as a response to aging but can be accelerated by unknown causes. Whereas rheumatoid arthritis is initiated by an inflammatory response which can lead to the destruction of articular cartilage, osteoarthritis is initiated by the destruction of or lack of repair of articular cartilage. This can lead to an inflammatory joint response, but rarely do the pathologic features of inflammation mimic those of rheumatoid arthritis. In addition, subsequent to ulceration of cartilage, new bone formation occurs at the margin of articular cartilage. These marginal osteophytes are the characteristic bony "spurs". Osseous changes also include cysts of varying sizes beneath the joint and remodelling of subchondral bone (1).

As with rheumatoid arthritis there are also variants of the osteoarthritis syndrome such as calcific tendonitis and bursitis which results in the most common cause from calcium deposits in the rotor tendons and inflammation of the bursae which are closed synovial sacs located at sites of friction between skin, ligaments, tendons, muscles and bones. These constitute the group of non-articular rheumatism (1).

Gout represents a group of genetic diseases and acquired disorders of purine metabolism or excretion that may be identified by hyperuricemia (increase in blood uric acid). Therefore gout is an heterogeneous collection of disorders, and different disease states leads to the common feature of persistent hyperuricemia. Also, hyperuricemia may occur following chemotherapy for malignancy disorders due to the destruction of tissue resulting in uric acid overload due to an increase in purines (1).

Uric acid is not toxic in its soluble form, but causes problems when precipitated as sodium urate at pH 7.4 or as uric acid in acidic urine. The precipitation of uric acid as sodium urate may occur in any tissue and results in tophi with tissue disruption and destruction. Its precipitation in the kidney can result in varying degrees of renal impairment. Its precipitation as microcrystals in synovial tissues and spaces leads to acute inflammatory arthritis also known as gouty arthritis (1).

Hyperuricemia and gouty arthritis have also been observed following heart transplantation (5).

Gout is therefore an inflammatory arthritis induced by urate crystals (monosodium urate crystals). The mechanisms by which these monosodium urate crystals (MSUC) lead to synovitis and joint destruction have not been completely elucidated. Nevertheless, eicosanoids have been implicated which are important mediators of inflammatory responses, and are produced in response to MSUC (6). Elevated concentrations of eicosanoids are found in gouty synovial fluid (7, 8), prostaglandin (PGE₂) which stimulates bone reabsorption (9) and hydroxyeicosatetraenoic acids are produced by synovial cells exposed to MSUC (10-14). Also, leukotriene B₄ is present which stimulates neutrophil infiltration (15). It appears that the rate limiting step in eicosanoid production is the production of phospholipase enzymes which cleave fatty acids from membrane phospholipids (16). These enzymes are produced by monocytes and neutrophils stimulated by MSUC via a phospholipase activating protein which can be inhibited by colchicine and indomethacin (16). Whether or not asymptomatic gout, in terms of the immunological mechanisms which precede rheumatoid arthritis and osteoarthritis has not been elucidated. In this respect it is interesting to note that hyperuricemia is not a disease but a risk factor for gout and coronary heart disease (17). In

addition, MSUC may be present in synovial fluid in the absence of hyperuricemia (1, 18, 19), therefore making the diagnosis of gout difficult.

5 Various treatments have been proposed for treatment of inflammatory complaints but they have only met with partial success and in this regard have not proved to be satisfactory as described in Sany (23). For example, in relation to rheumatoid arthritis various therapies have been proposed which include 10 either slow acting drugs which act mainly but not exclusively on macrophages (i.e. gold salts) or on T CD4+ cells (i.e. D penicillamine) or immunostimulating agents (i.e. Lemamisole) or immunosuppressive drugs such as alkylating agents and antimetabolites. Aspirin 15 as an analgesic and NSAIDS (non steroidal anti-inflammatory drugs) have also been proposed as well as methotrexate as described in Kremer (3). All these therapies have associated side effects of various severity.

20 Reference may also be made to references (19), (16), (24), (18), (25), (26), (17), (27), (4) and (5) which also discuss this subject.

The treatment of gout may also be problematic. The use of the anti-inflammatory agents, phenylbutazone 25 or indomethacin can result in headaches and gastrointestinal symptoms (nausea, vomiting, abdominal pain and diarrhoea), but less than that experienced with colchicine which may also induce leucopenia. Glucocorticoid or ACTH give variable responses and 30 there is a high incidence of rebound effects (1).

Reference may be made to prior art which includes Japanese patent JP1157915 which described a superoxide dismutase (SOD) inducer containing celery or lovage extracts which may be extracted by distillation 35 with steam or with organic solvents by a batch or reflux method. The extract is used at any concentration or also as a dried substance. A

surfactant or lactose may be added.

French patent No. 2611502 refers to an extract of roots from the plant family *Umbelliferae* which has diuretic, cardiovascular, analgesic and anti-inflammatory activity. The roots are extracted with an alcoholic solvent and dried to a powder or used as a preservative.

German patent No. 3638484 refers to a plant extract from parsley in an aqueous or alcoholic medium for use as a medicinal anti-lice shampoo.

Japanese patent JP1257155 refers to anti-oxidant food containing a powdered extract of a plant belonging to the *Umbelliferae* family.

Japanese patents JP57056416 and 587058327 refer to mouthwash compositions containing an alcoholic extract of celery.

Russian patent SU639552 refers to an alcoholic extract of parsley juice as an obstetric composition.

Reference may also be made to Japanese patent specifications 63-90505 and 63-90506 which relate to a novel polysaccharide derived from rhizome of *Bupleurum falcatum* belonging to the *Umbelliferae* plant family and effective as a therapeutic agent in relation to autoimmune diseases such as arthrorheumatism, thyroiditis and chronic nephritis.

Extracts from the seeds of the celery plant *Apium graveolens* (family *Umbelliferae*) have been asserted to have pharmacological properties. These range from carminative, diuretic, emmenagogic (an agent that facilitates menstrual discharge) and antiseptic effects. In addition such extracts have also been asserted to be useful in the treatment of bronchitis, asthma, liver and spleen disorders, inflammation, rheumatism, arthritis and gout (28, 29, 30). These therapeutic properties of the celery seed extracts are generally attributed to the essential oils in the extracts. These essential oils are in fact the

volatile oils that constitute 1%-2% vol/weight of seed, which can be obtained by steam distillation (31). The composition of the essential oils has been established by gas chromatography as described in McLeod et al (32) and Salzer (33). The main constituents of the oil are the terpene hydrocarbons limonene and β -selinene which together constitute about 80% of the total oil. The remainder is made up of other terpenes and compounds which give the characteristic odour of celery oil e.g. the butyl phthalides (31). Scientific data has demonstrated that both aqueous and 80% ethanol extracts of the celery stalk have anti-inflammatory properties as described in Lewis et al (34) and Al-Hindawi et al. (35).

In relation to conventional alcoholic extraction of celery, one method is to extract celery seed with 95% ethanol until all ethanol soluble celery components have been extracted. After filtration to remove particulate material, the soluble components are then used as the therapeutic agent based on the content of volatile oils.

SUMMARY OF THE INVENTION

An object of the invention is therefore to provide therapeutic agent(s) for the treatment of inflammatory diseases or complaints generally and/or also in relation to diseases, illnesses or complaints caused by uric acid accumulation in the body.

The therapeutic agent(s) of the invention may also be used for treatment of endometriosis, which refers to the growth of cells in the lining of the womb in the body cavity which causes an inflammatory response.

The therapeutic agent(s) of the invention include compounds of general formulae I, II, III, IV, V, VI, VII, VIII, IX, X, XI and XII reproduced hereinafter in FIG 1 as well as mixtures thereof. The therapeutic agent(s) of the invention may also include

compositions containing one or more of the above compounds which may also contain one or more fatty acids.

In the formulae given in FIG 1, R_1 and R_2 are
5 alkyl chains preferably having 8-22 carbons. Suitably R_1 and R_2 may include structures of the formula -
 $CH(CH_2)_n-$ or $=CH(CH_2)_n-$ or isomers of these structures including branched chain structures. The value of n
may be any whole number up to 30 but preferably n has a
10 value of from 6 to 20. R_1 and R_2 may contain an even or odd number of carbon atoms and may be the same or different.

R_1 and R_2 may terminate with a terminal group T which may be methyl, ethyl, hydroxyl, carboxylic acid,
15 alcohol or derivatives of carboxylic acids or salts of carboxylic acids. R_1 and R_2 may also contain single or double bonds in a conjugated or non-conjugated form.

R_1 and R_2 may also be alkyl chains optionally substituted with any suitable substituent which may
20 include amino, hydroxyl, sulphonyl, nitro, oxo, alkoxy, halogen, substituted amino with the substituent(s) being alkyl or substituted sulphonyl wherein the substituent(s) may be alkyl.

Optional substituents S_1 , S_2 , S_3 and S_4 may
25 also be selected from amino, hydroxyl, sulphonyl, nitro, oxo, alkoxy, halogen, substituted amino wherein the substituents may be alkyl groups or substituted sulphonyl wherein the substituent(s) may be alkyl.

The bond indicated in phantom indicates the
30 presence of an optional bond at the positions indicated where possible due to valency requirements.

Compounds of the general formula II may be formed from phthalic anhydride by reaction with a
suitable Grignard reagent $R Mg X$ where X is halide and R
35 is alkyl. Subsequently compounds of general formulae I and III may be formed by initial reaction of compound II with an alkyl halide and subsequent reaction with an

alcohol. Alternatively compound II may be reacted with an alcohol followed by a dehydration reaction with an acid. Compounds of general formula IV may be produced from phthalic acid with an appropriate alkylating agent such as a Grignard reagent.

Compounds of general formulae VI may be produced by reaction of naphthoquinone with an appropriate alkylating agent such as a Grignard reagent at the 2 and 3 positions to produce a compound formula VI followed by subsequent dehydrogenation reaction as described above at the relevant locations to produce compounds of formulae V and VII.

Compounds VIII and IX may be produced by a Friedel Crafts reaction involving alkylation at the relevant position where the alkyl moiety is a branched structure.

Compounds X, XI and XII may be produced by substitution of an -NR group instead of the oxygen at the relevant position by reaction of ammonia or alkylamine with the corresponding I, II or III compound.

The plant extracts of the invention may be prepared by a process which includes the steps of:

- (i) contacting plant material with an organic solvent which may extract soluble components of the plant material to produce a solvent extract;
- (ii) separating the mixture of plant material and solvent so as to remove particulate material therefrom;
- (iii) concentrating the extract by removing the solvent.

The invention also includes within its scope plant extracts prepared in accordance with the abovementioned process.

The invention also includes within its scope plant extracts which may contain one or more of the

abovementioned compounds and in particular plant extracts of the genus *Umbelliferae*, celery (*Apium graveolens*) parsley (*Petroselinum hortense*) and dill (*Anethum graveolens*).

5 It will be appreciated, however, that such compounds may also be located in plants of the genus *Solanum*.

 The plant extracts may also be derived from the leaves of plants as well as the stalks but
10 preferably use is made of plant seeds as starting material.

 In relation to celery seed, the varieties of suitable celery seed includes the French, Chinese and Indian species. The Indian variety of celery seed
15 however is preferred.

 The celery seed is preferably crushed to a powder. This can be achieved by any one of a number of methods. For a relatively low mass of celery seed, a mortar and pestle is suitable for crushing.

20 Alternatively, the plant material may be crushed or ground in a ball mill, plate mill or other suitable means for comminuting the plant material. Initially the celery seed which may have an average dimension of 1.5 x 1.55 mm and a thickness of 0.5 mm
25 may be comminuted to provide finely divided particles of an average diameter of 0.1 - 0.2 mm.

 Following crushing, the crushed seed may be suspended in a suitable solvent selected from a group comprising alcohol, acetone, methylene chloride,
30 benzene, chloroform, glycols including propylene glycol, ether and appropriate organic oils. Preferably, the solvent is an alcohol. A preferable alcohol is ethanol. A suitable concentration of ethanol is 95% - 98% (v/v). The preferred ratio of
35 seed to ethanol is 1 kg of seed in 16 litres of ethanol but the ratio may be as high as 1 kg of seed in 5 litres of ethanol.

However, in accordance with this invention while it is possible to use alcohol of 95-98% concentration it is more effective in the process of this invention to use absolute alcohol or alcohol re-
5 distilled from 95-98% concentration. Thus the alcohol may have a preferred concentration of 99.99%.

The suspension can be subjected to slow agitation until the extractable components in the celery seed have been fully extracted with the chosen
10 solvent. The temperature of incubation may vary but the preferred temperature is room temperature. Higher incubation temperatures may be used. The incubation may occur in a closed system such as tanks of varying capacities. The incubation time may vary depending
15 upon the celery seed condition but a preferable time may be up to eight days.

Particulate plant material is subsequently removed. Preferably the particulate plant material is removed by filtration and/or sedimentation although
20 centrifugation may also be used. Suitably, the extract is filtered with filters of a pore size that have a range of from 0.2 to 10 microns and more suitably 4-5 microns. Two or more filters may be used in series. The filtering process may be assisted by the
25 application of positive pressure or the use of a vacuum.

The extract with the particulate plant material removed may then be concentrated to remove solvent and water if present, leaving a residue of non-
30 volatiles. Solvent may be distilled from the extract with the use of heat, vacuum and/or pressure. Where the solvent is ethanol, the temperature of distillation is variable but preferably 50-60°C.

In a preferred form of the invention, an
35 initial extract may be prepared which is an alcoholic extract which is a 1 in 10 concentrate (e.g. 1 kg of alcoholic extract is prepared from 10 kg of seed).

The alcoholic extract may be further concentrated under vacuum so that there is a 50% weight loss due to the removal of ethanol and water to provide a viscous liquid or concentrate. Suitably 10 kg of seed provides 0.5 kg of concentrate or a 1 in 20 concentrate.

In accordance with the present invention it is believed that compounds of the abovementioned formulae and in particular butylphthalide, sedanenolide and sedanolide (in both cis and trans forms otherwise known as essential oils which are mainly derived from plants) mimic the physiological response of essential fatty acids and also their prostaglandin analogues. Such fatty acids comprise the n-6 series derived from linoleic acid and the n-3 series derived from α -linolenic acid as discussed in Horrobin and Manku pages 22-24 entitled "Clinical Biochemistry of EFA" located in Essential Fatty Acids Pathophysiology and Roles in Clinical Medicine (36).

When the therapeutic agent(s) of the invention are administered to subjects suffering from inflammatory complaints the ingestion or injection of the abovementioned compounds result in an elevation of the same or similar fatty acids in the blood resulting in a reduction of traumatic pain. Essential fatty acids are involved in the regulation of all body functions.

The plant extract of the invention will suitably be marketed in the form of a powder which may be dried under vacuum and which may also contain dicalcium phosphate or other suitable fillers.

A suitable composition for use in the invention may include one or more compounds of general formulae I to XII described herein in FIG 1 together with 5-95% by weight of filler. More preferably the filler is included in said composition on a basis of at least 50% by weight and most preferably the filler is

included in said composition of at least 75% by weight.

In relation to the fillers that may be used in combination with the therapeutic agent of the invention, such fillers may include silica, dextrans, glucose or other suitable carbohydrate. However, dicalcium phosphate may be preferred.

Spectrophotometry of Ethanol Extract

Essential oils are soluble in alcohol and absorb in the U.V. region of 190-214 nm (37). Therefore in order to demonstrate the presence of essential oils in the alcohol extract U.V. spectrophotometry was carried out.

Example I demonstrates that the maximum absorption of the extract in 80% alcohol (i.e. ethanol) is at 200 nm when diluted 25,000.

High Performance Liquid Chromatography (HPLC) of the Ethanol Extract

Based on the HPLC methodology described in Applications of HPLC in Biochemistry by Fallon A, Booth R F G and Bell L D, pages 193-196 (37), Example II demonstrates the presence of three (3) major peaks of essential oils in the extract and their associated profile. The peaks are A, B and C.

Example III describes preparative HPLC conditions for the isolation and purification of the essential oil peaks A, B and C.

From the isolation of the pure compounds the weight was found and hence the concentration of these essential oils in the ethanol extract and concentrate can be determined.

Spectrophotometry of the Essential Oils (A, B and C) (Example IV)

Spectrophotometry of the pure essential oils (A, B and C) in 80% ethanol demonstrated that there were no other absorption peaks in the visible region and only the characteristic U.V. profile as previously shown in Example I. That is, a peak of absorption at

280 nm and maximum absorption at 191-192 nm in this pure form (Example IV).

Mass Spectrophotometry Nuclear Magnetic Resonance and Infrared Analysis (Example V)

5 Mass Spectrophotometry (MS), Nuclear Magnetic Resonance (NMR) and Infrared Spectrophotometry (IR) have identified the three peaks fatty acids/oils as n-butylphthalide, sedanenolide and sedanolide (Example V).

10 Extract Formulation

From pilot experiments it was determined that an 80% ethanol solution, containing 34 mg dry weight/ml of the concentrated ethanol extract, would be used for biomedical studies for the relief of rheumatic pain.

15 The HPLC profile of 34 mg/ml of the ethanol extract is shown in Example VI. Thus the ratio of peaks A, B and C to each other can be calculated from their peak heights.

20 Thus B/A = 9.50
 C/A = 26.25
 C/B = 2.76

Hence from the ratios of B/A and C/A, the concentration of these peaks in terms of total solids in the extract and in the diluted extract (34 mg/ml) can be calculated.

25 Thus in terms of the diluted ethanol extract of 34 mg/ml: 1 : 9.5 : 26.25

30 A = $\frac{1}{36.75}$ x 34 = 1.008 mg

B = $\frac{9.5}{36.75}$ x 34 = 8.789 mg

35 C = $\frac{26.25}{36.75}$ x 34 = $\frac{24.286}{34.083}$ mg

40 In Example III, from a known concentration of peak C (peaks A, B and C are similar) the concentration of the fatty acids/oils A, B and C and region D were calculated using the peak heights shown in Example VI.

15

Thus A = 0.5 mg/ml
 B = 7.5 mg/ml
 C = 20.5 mg/ml

5 28.5 mg/ml

The "D" region must be

34 - 28.5 mg = 5.5 mg/ml

Thus the ratio of A, B, C, D is -

10 1:15:41:11

Plasma Fatty Acids

Since many fatty acids are naturally occurring in human blood, samples of blood were taken before and after the ingestion of an 80% ethanol tincture containing 34 mg/ml of extract and in a capsule form containing 68 mg of extract. The results were similar. In both cases the blood level of these three (3) particular fatty acids/oils and other fatty acids were elevated and then decayed over 3 hours to levels less than that at zero time i.e. before the extract was taken. In addition the blood plasma co-chromatographed with the pure fatty acids/oils A, B and C, demonstrating identity between the fatty acids/oils in the blood and those in the extract (Example VII).

25 Pilot Study

A pilot study was carried using the McGill Pain Questionnaire: 15 volunteers, age 33-83 years with medically diagnosed rheumatic pain for 10 ± 8.5 years that was continual or intermittent. The volunteers were assessed at four consecutive 3 weekly intervals, after which they discontinued taking the extract and were allowed to return to the study of their own volition. At 12 weeks, the volunteers showed a statistical decrease in transformed data of usual pain ($t = 4.2$ $p < 0.001$) and present pain ($t = 5.23$, $p < 0.001$). The decrease in pain was time-dose dependent ($r = -0.90$, $0.001 < p < 0.01$). Also usual pain and present pain were negatively correlated ($r = -0.90$, $0.001 < p < 0.01$). Similarly there was a significant

statistical decrease in the number of usual and present body areas experiencing pain ($p < 0.001$) which were correlated with the decreases in usual pain ($0.001 < p < 0.01$) and present pain ($0.02 < p < 0.05$). Upon re-entry into the study, the volunteers average pain level was 70% of the original pain level. The time delay for re-entry into the study was 2-57 days. Decreases in usual pain, present pain, usual and present body areas experiencing pain followed the same pattern as the first part of the study. Standard blood biochemistry and haematology was normal.

Dose Response

Based on the pilot study of pain relief from arthritic and rheumatic pain, from the correlations previously summarised, and from Example III i.e. present pain is correlated with usual pain and body areas experiencing pain over 12 weeks. Then the amount of extract consumed at 3, 6 and 12 weeks can be plotted against decrease in present pain (FIG 34). FIG 35 shows the extrapolation of the graph in terms of extract to be consumed to achieve zero pain.

Thus:

Extract was 34 mg/ml in 80% ethanol, 2 ml/day
= 68 mg of extract/day
= 1428 mg/3 weeks
= 2856 mg/6 weeks
= 5712 mg/12 weeks

Extrapolation gives 11400 mg to achieve zero pain.

1. Over 12 weeks this dose is 958.33 mg/week or 136 mg/day.
2. Thus to achieve zero pain relief in 1 week this dose would be $958.33 \times 12 = 11500$ mg/week
or 1642.85 mg/day
or 821.42 mg/bidaily.

This is considered to be the maximum dose per day 1642.85 mg of a 1 in 20 concentrate of celery seed

extract.

The minimum dose is that used in this study which is 68 mg/day of a 1 in 20 concentrate of a celery seed extract.

5 From the Extract Formulation page and Example VI, the ratio of the peaks A, B and C were derived. The concentration of these peaks in an 80% ethanol solution of the extract of 34 mg/ml was then calculated. Thus the same calculations can be carried
10 out for the maximum single dose of 821.42 mg.

$$\therefore A = 22.35 \text{ mg}$$

B = 212.34 mg

C = 586.73 mg

821.42 mg

Similarly as the peaks A, B and C from the pure compounds can be determined Example VI and the D region. Hence the concentration of the fatty acids/oils A, B, C and D region can be calculated.

Thus A = 12.1 mg/ml

B = 181.2 mg/ml.

C = 495.3 mg/ml

788.6 mg/ml

D region is $821.42 - 788.6 = 32.82 \text{ mg/ml}$.

EXAMPLES

EXAMPLE I

30 U.V. and Visible Spectrophotometry of the
 Ethanol Extract

Method

Measuring wavelength range 900 - 185 nm

Measuring mode (ABS)

35 Photometric scale (ABS 0 - 1)

```

Response time . . . . . 1 sec

```

Slit width 2 nm

Wavelength scale expansion 20 nm/cm

Scan speed 60 nm

40 (1) Set absorption cells filled with solvent on

the sample and reference sides. (quartz cells used)

(2) Set up measuring conditions as follows:

```

5      Start wave length . . . . . 900 → GO TO λ
      Measuring mode . . . . . ( ABS → Depress )
      Response time . . . . . 1 → RESPONSE
      Slit width . . . . . 2 → SLIT
      Wavelength scale expansion 20 → CHART FORMAT
      Scan speed . . . . . 60 → SCAN SPEED
10     Recording mode . . . . . RECORDING → OFF
           Set to SEQ for obtaining spectrum
           without background correction.
      Light source . . . . . LAMP SELECT → AUTO

```

15 Photometric scale 0 → (SCALE) → 100 → (SCALE)
(0 → (SCALE) → 1 → (SCALE))
Correcting wave-
length . . 900 → (BACKGROUND) → 185 → (BACKGROUND
(CORRECTION) (CORRECTION))
20 ON ($\lambda_1 - \lambda_2$) nm lamp lights up.

(3) Depress the **SCAN** key to start background correction. The UNDER CORR lamp lights up. Background correction is executed in a wavelength range from 900 to 185 nm.

25 From the resulting visible and UV spectrophotometry diagram as shown in FIG 2, it was noted that the celery seed extract of the invention had a maximum absorption at 200 nm when diluted 1 in 25,000.

30 EXAMPLE II

High Performance Liquid Chromatography of the Ethanol Extract (Analytical)

H.P.L.C. was carried out in relation to the ethanol extract using a Waterman single pump and a C₁₈ analytical column. The buffer was 48% acetonitrile, 52% water, pH 7.0, flow rate 0.5 ml/min, chart speed 5mm/min, absorption 203 nm and a scale of 0.01 full

deflection.

FIG. 3 refers to 1 μ l of the ethanol extract at a 1 in 10 dilution. The essential oil peaks of interest are A, B and C.

5 We also attach FIGS 4-6 to demonstrate the absorption range of the essential oils A, B and C.

FIG 4 refers to 1 in 200 dilution of the ethanol extract showing a loss of peak A at 252 nm and a reduction in the size of peak B.

10 FIG 5 refers to a 1 in 50 dilution of the ethanol extract showing a loss of peak A and a reduction in peak B and C at 280 nm.

FIG 6 refers to a 1 in 50 dilution of the ethanol extract showing the loss of peaks A and B and almost the complete loss of peak C at 340 nm.

15 This demonstrates the peaks of the extract in that the absorption U.V. profile (Example II) is due to the essential oils and not to any contaminants in the ethanol extract. D as shown in FIGS 4-6 corresponds to that part of the absorption trace which does not include peaks A, B and C.

EXAMPLE III

High Performance Liquid Chromatography

(Preparative)

25 The extract was diluted 1 in 10 with 48% acetonitrile and 52% water pH 7.0. The resultant black precipitate was then extracted 1 in 20 with hexane. The hexane was removed under vacuum in a rotary evaporator. This gives a green oil. This was then applied to a C₁₈ preparative HPLC column using 48% acetonitrile 52% water as buffer.

30	Flow rate	5 ml/min
	Scale	0.4 full deflection
	Chart speed	5 mm/min
35	Absorption	203 nm

The first isolation of the 3 peaks A, B, C FIG. 7 demonstrates that peaks B and C would need to be

rechromatographed. Thus FIG 8 shows the
rechromatographed peak C and FIG 9 shows
rechromatographed peak B. Thus the three peaks (ABC)
have been purified. The solutions of peaks A, B and C
5 were then concentrated under reduced pressure in a
rotary evaporator to dryness and weighed and
redissolved in 80% ethanol for analytical HPLC as
previously described in Example II.

EXAMPLE IV

10 Spectrophotometry of Fatty Acid Peaks A, B and C

Spectrophotometry in both the visible and U.V.
region was carried out as previously described in
Example I.

15 There was no absorption in the visible region
of the spectrum. In the U.V. region peak A had maximum
absorption at 190 nm peak B 191 nm and peak C 192 nm.
This is shown in FIGS 10-12.

EXAMPLE V

20 Mass Spectrometry, Nuclear Magnetic Resonance and Infrared Spectrometry

Example V relates to further molecular
characterization of the peaks A, B and C previously
described. This is shown in FIGS 13-18. More
25 particularly, FIGS 13-18 represent the simultaneous
characterization of a sample of peak A, peak B and peak
C using Mass Spectrophotometry (MS), Nuclear Magnetic
Resonance (NMR) and Infrared Spectrophotometry (IS).
FIG 13 is a MS analysis of peak A. The butylphthalide
30 structure is evident. FIG 14 illustrates NMR of the
same peak A, confirming the conclusions drawn from MS.
This shows that approximately 70% sedanenolide and 30%
n-butylphthalide are present.

FIG 15 is a MS analysis of peak B. Again, the
35 butylphthalide structure is evident. This is confirmed
in FIG 16, by the NMR of the same peak B, that shows n-
butylphthalide is present. FIG 17 is a MS analysis of

peak C. This shows that sedanolide is present. Further structural analysis using Infrared Spectrophotometry as shown in FIG 18 confirms the conclusions derived from the MS.

5

EXAMPLE VI

Analytical HPLC of the Alcohol Extract diluted with 80% Ethanol to give a solids concentration of 34 mg/ml

Analytical HPLC was performed as previously described in Example II.

10

FIGS 19-22 demonstrate the linear relationship of the peak heights to volume of sample used. The sample was the ethanol extract diluted to give 34 mg/ml which was diluted 1 in 10 for HPLC analysis.

15

Thus in FIGS 19-21 peak 1 corresponds to 2 μ l of the sample, peak 2 corresponds to 1 μ l of the sample, peak 3 corresponds to 4 μ l of the sample, peak 4 corresponds to 3 μ l of the sample and peak 5 corresponds to 5 μ l of the sample.

20

From this data a calibration curve as shown in FIG 22 could be constructed and the concentration of the relevant components determined.

EXAMPLE VII

Blood Plasma levels of Fatty Acids before and after ingestion of the Extract

25

Analytical HPLC was carried out as previously described (Example II).

30

Blood samples were taken at zero time and at various time intervals thereafter following the ingestion of the extract. Blood samples of 5.0 ml were taken by venipuncture into EDTA-coated tubes and centrifuged to obtain the plasma.

35

The plasma was then diluted 1 in 2 with 80% ethanol to precipitate proteins and leave fatty acids in solution. A 0.1 ml aliquot of plasma was made up to 0.5 ml with 80% ethanol. Then 0.5 ml of 48% acetonitrile was added, giving a total dilution factor for the plasma of 20. Then 1.0 μ l was taken for

analysis.

FIG 23. Subject to zero time showing the presence of the three naturally occurring peaks in the plasma (A,B,C). After taking the ethanol extract (340 mg) the C peak was off scale, hence a 20 fold dilution had to be used.

FIG 24. Subject 1 zero time 20 fold dilution of plasma. Peak C remains but peaks A and B are not detectable at this dilution.

FIG 25. Subject 1 20 min after ingestion of the extract shows an 108% increase in peak C.

FIG 26. Subject 1 70 min after ingestion of the extract shows a 33% reduction in peak C.

FIG 27. Subject 1 3 hours after ingestion of extract shows the absence of peak C.

FIG 28. Subject 2 zero time and 35 min after ingestion of 136 mg of extract. Peak A has increased 50%, Peak B has increased 60% and Peak C has increased 62%.

FIG 29. Subject 3 zero time and 1.0 hours after ingestion of 136 mg of extract. Peak A has increased 50%, Peak B has increased 68% and Peak C has increased 85%.

FIG 30. Subject 4 zero time and 85 min after ingestion of extract. Only Peaks B and C are present which became reduced by 50%.

FIG 31. Subject 5 zero time and 3 hours after ingestion of the extract. Only peaks B and C are present which become reduced by 40%.

These results show that although the zero time concentrations of the peaks A, B and C vary between individuals, they follow the pattern of increase after ingestion of the extract and by 3 hours decrease below that of zero time.

The plasma peaks A, B and C are similar if not identical to those in the extract in terms of retention time on a C₁₈ column. Also the pure compounds in the

extract and plasma FIG 32, co-chromatographic FIG 33.

EXAMPLE VIII

Pilot Biomedical Study for pain relief in
Rheumatic Pain

5 1. Statistical Considerations

1.1 Rheumatic Illness

Rheumatism represents over 100 types of rheumatic illnesses and the syndromes can present manifestations of and/or be associated with other diseases. This complexity therefore makes the classification of rheumatic illness, its treatment and diagnosis difficult. Thus diagnosis has been considered as a variable, which has been controlled/allowed for by accepting volunteers into the study diagnosed by individual medical specialists and general medical practitioners and self diagnosis where proof was evident.

1.2 Pain

The major common factor with rheumatic illness is pain from affected body areas. Therefore the standard McGill Pain Questionnaire was used, which is designed to provide quantitative measures of clinical pain, the pain rating index, that can be assessed statistically and for individual comparisons. In addition, the questionnaire was structured such that the same questions were asked in different ways in order to cross-check responses by statistical analysis.

1.3 Placebo Control

Pain is a variable in that it may be remittent or continual. Therefore assessment of usual pain and usual body areas experiencing pain as well as present pain and present body areas experiencing pain at interview was measured. Therefore, if there is an absence or limited placebo effect, then, usual pain and usual body areas experiencing pain should significantly decrease with time and be correlated. That is, a placebo does not act retrospectively. Similarly, a

significant decrease in usual pain with time should be correlated with a significant decrease in present pain. Also, a significant decrease in present pain with time should be correlated with a significant decrease in present body areas experiencing pain. In addition, a significant decrease in usual body areas experiencing pain with time should be correlated with a significant decrease in present body areas experiencing pain.

1.4 Controls

Due to the variables discussed in 1.1 and 1.3, a control group of volunteers would be difficult if not impossible to establish. This may be circumvented by volunteers acting as their own control. This is achieved by expressing pain during the study as a ratio to that experienced at the start of the study.

1.5 Efficacy

Efficacy is in essence a measure of a product's performance claims. Thus products may have a low to high efficacy. This means that for low efficacious products, where few subjects respond to treatment, large numbers of subjects are required for the study to define those who will receive the most benefit. With high efficacious products, few subjects are required to demonstrate efficacy.

1.6 Study Design

Consideration was given to cross-over studies (one group) or double cross-over (2 groups) whereby the volunteers at one stage take a placebo and at another stage take the active product. However, since a placebo with the same taste as the active product is not known to exist, and the duration of and mode of action of the active component(s) is not known (wash-out), cross-over points cannot be established. Thus a longitudinal study was designed in which the first part of the study is repeated when volunteers request to return to the study after cessation of taking the extract. In this way, together with the Statistical

Considerations (section 3), a placebo effect is controlled and efficacy can be determined.

5 A total of 15 volunteers were assessed using the McGill pain questionnaire at the start of the study, and a 5ml blood sample was taken for blood biochemistry analysis. The volunteers were then given a 3-week supply of the extract in tincture form ie. in 80% ethanol at 34 mg/ml. One (1.0 ml) 2 x day taken in a small amount of water.

10 At the end of 3 weeks, the volunteers were assessed as previously described and the procedure was repeated for another 3 weeks (total 6 weeks). The volunteers were then given a 6-week supply of the extract and a follow-up assessment was conducted at the
15 end of this 6 weeks (total 12 weeks). The volunteers then stopped taking the ethanol extract, and if they wished to return to the study, they notified the field officer and the first part of the study (2x3 weeks) was repeated.

20 2. Results of 12 weeks (0, 3, 6, 12 weeks)

The cohort is composed of 15 volunteers that in the majority of cases (93%) have been medically diagnosed as having arthritic or rheumatic pain, including osteoarthritis, osteoporosis or gout. The
25 pain has been present for approximately 10 years in a remittent or continual form, and the majority (73%) of the volunteers have attempted to use various methods to achieve pain relief. The pain and lack of joint mobility has prevented the carrying out of household
30 duties, hobbies and activities involved in employment.

The general perception of pain by the volunteers is similar to that experienced with rheumatic/arthritic pain. This indicates that these volunteers have had experience of pain over a long
35 duration.

The mean age of the volunteers places them in the higher incidence group for arthritic rheumatic

disorders. The current medication, if applicable, for each of the volunteers is shown in Table 1.

Interpretation

5 The correlation between present pain intensity and the sum total (Σ) of volunteers over 12 weeks as shown in Table 2 and FIG 34 is statistically significant. This would occur by chance in less than 1 in 100 cases. This demonstrates that present pain intensity for the volunteers continues to decrease over
10 12 weeks using the ethanol extract (time dependent efficacy).

 The statistical analysis is biased against the efficacy of the ethanol extract in that volunteers without pain have not been considered.

15 Interpretation

 In relation to interpretation of the data shown in FIG 35 and Table 3, there was a significant decrease in the total usual pain index at 3 weeks and 6 weeks relative to each other and week 0, whereas at 12
20 weeks there was no further decrease.

 Present pain also showed a significant decrease at 3 weeks which was similar to that at 6 weeks with a further significant decrease at 12 weeks relative to that at 6 weeks. The probability that this
25 would occur by chance alone is less than 1 in 1000 cases.

 There is also a significant correlation between the decrease in usual and present pain index. Thus as usual pain decreases over 12 weeks, so does
30 present pain. This would occur by chance alone in less than 5 in 100 cases.

Interpretation

 The Pain Index for each case at 3, 6 and 12 weeks has been transformed by expressing it as a ratio
35 relative to that at the start of the study, 0 week as described in Table 4. Thus each volunteer is acting as their own control: Pain Index at 3, 6 or 12 weeks

Pain Index at 0 week

Interpretation

In relation to interpretation of the data shown in Table 5, this will be described hereinafter.

5 Usual Pain

The parametric analysis by using the 't' test demonstrates that at 3 weeks there are 4 non-responders and that the mean transformed pain index is not significantly different from that at week 0. At 6 weeks the non-responders had responded and there was now a significant decrease in the transformed mean pain index which is similar to that at 12 weeks. This would occur by chance alone in less than 1 in 1000 cases.

If the non-responders and responders without pain at 3 weeks, and the responders without pain at 12 weeks are not considered, then there is a significant decrease in the mean residual pain index at 3 weeks and 12 weeks. This would occur by chance by less than 1 in 1000 cases.

20 Present Pain

There was a significant decrease in the mean transformed pain index at 3 weeks which is similar to that at 6 weeks and 12 weeks. This would occur by chance in less than 5 in 100 to less than 1 in 1000 cases. If the responders without pain at 3, 6 and 12 weeks are not considered, there is still a significant decrease in the mean residual pain index. This would occur by chance in less than 1 in 100 to less than 1 in 1000 cases.

30 Interpretation

In relation to interpretation of the data shown in FIG 36 and Table 6, there was a significant decrease in the mean number of usual and present body areas experiencing pain at 3 weeks which was similar to that at 6 and 12 weeks. This would occur by chance alone in less than 1 in 1000 cases.

There is also a significant correlation

between the decrease in the mean number of usual and present body areas experiencing pain over 12 weeks. This would occur by chance in less than 1 in 1000 cases.

5 Interpretation

In relation to interpretation of the data shown in FIG 37 and Table 7, the correlation between usual body areas experiencing pain and the usual pain index is significant. This would occur by chance in
10 less than 1 in 100 cases. This demonstrates that as the number of usual body areas experiencing pain decreases, so does the usual pain index decrease.

Interpretation

In relation to interpretation of the data
15 shown in FIG 38 and Table 8, the correlation between present body areas experiencing pain and present pain is significant. This would occur by chance in less than 5 in 100 cases. This demonstrates that as the number of present body areas experiencing pain
20 decreases, so does the present pain index.

3. Follow-up of volunteers upon re-entry to the study after cessation of ethanol extract treatment at 12 weeks

A total of seven volunteers have been lost to
25 follow-up.

Three volunteers discontinued without reason. One volunteer had recurrence of a medical problem and discontinued. One volunteer did not comply because of travelling interstate. Two volunteers were still free
30 of pain at 57 days and did not wish to re-enter the study. By 57 days post-cessation of the ethanol extract treatment, eight volunteers had returned to the study.

After cessation of the ethanol extract at 12
35 weeks, the volunteers (eight) notified the field officer that they wished to start taking the ethanol extract again. The pain questionnaire was completed

and this is referred to as 0 week. The present pain at 0 week was expressed as a percentage of the original present pain (original 0 week at the start of the study). The number of days taken for the pain to increase since cessation of the ethanol extract was noted and expressed as a ratio of the number of days before requesting to start taking the ethanol extract once more. This data is presented in FIG 39 and Table 9.

10 Interpretation

If the development of present pain becomes approximately equal to the original pain, the ratio is 0.5. That is, the volunteers tolerate pain for an equal amount of time that it took to develop the pain. The lower the percentage of the present pain, relative to that of the original pain at the start of the study, then the time delay in re-entering the study is greater than the time taken to develop that pain level.

This demonstrates that the request to re-enter the study is not based on a placebo effect.

Mean time for increase in pain 23 ± 17.4 days, range 9-57 days.

Mean time to re-enter study 34 ± 15.5 days, range 9-57 days.

Ratio = 0.68.

Thus, on average, volunteers will tolerate 70% of the original pain experience before re-entering the study.

The number of volunteers without pain leaves insufficient data for parametric analysis. Therefore, non-parametric analysis has been performed.

Pain upon re-entry into study

Interpretation

In relation to interpretation of the data given in Table 10, the eight volunteers' usual and present pain and usual and present body areas experiencing pain at 12 weeks prior to cessation of the

ethanol extract treatment are significantly decreased relative to re-entry into the study (0 week). Thus there has been significant increase in pain and body areas experiencing pain following cessation of the ethanol extract treatment. This would occur by chance alone in less than 5 in 100 to less than 1 in 1000 cases.

Follow-up after re-entry into study

Interpretation

10 In relation to interpretation of the data given in Table 11, there is a significant decrease in usual and present pain at 3 weeks and a further significant decrease at 6 weeks. This would occur by chance in less than 1 in 1000 cases.

15 There is a significant decrease in usual and present body areas experiencing pain at 3 weeks. This would occur by chance in less than 1 in 1000 cases.

At 6 weeks, there is no further decrease in usual body areas experiencing pain, whereas there is an increase in present body areas experiencing pain, but this is still significantly less ($p < 0.001$) than that at week 0.

20 The significant decrease in pain and body areas experiencing pain follows the same pattern as that for the first part of the study. The increase at 6 weeks in present body areas experiencing pain, which is still significantly less than that at 0 week, demonstrates the control of breakthrough pain by use of the ethanol extract.

30 Interpretation

In FIG 40 and Table 12, there is shown a graphical representation of usual pain of the eight volunteers from the first part of the study (0, 3, 6 and 12 weeks) and re-entry into the study (0, 3 and 6 weeks). This represents the statistical analysis of the McGill Pain Rating Index for usual pain.

35 Interpretation

In FIG 41 and Table 13, there is shown a graphical representation of present pain of the eight volunteers from the first part of the study (0, 3, 6 and 12 weeks) and re-entry into the study (0, 3 and 6 weeks). This represents the statistical analysis of the McGill Pain Rating Index for present pain.

Interpretation

In FIG 42 and Table 14, there is shown a graphical representation of usual body areas experiencing pain of the eight volunteers from the first part of the study (0, 3, 6 and 12 weeks) and re-entry into the study (0, 3 and 6 weeks). This represents the statistical analysis of the McGill Pain Rating Index for usual body areas experiencing pain.

Interpretation

In FIG 43 and Table 15, there is shown a graphical representation of present body areas experiencing pain of the eight volunteers from the first part of the study (0, 3, 6 and 12 weeks) and re-entry into the study (0, 3 and 6 weeks). This represents the statistical analysis of the McGill Pain Rating Index for present body areas experiencing pain.

4. Blood Biochemistry

In regard to the data shown in Table 16 which represents blood plasma biochemistry analysis, the blood samples were non-fasting and collected in EDTA tubes which limited the scope of the assays. Nevertheless, initial plasma samples can be compared with those samples taken at the time the volunteers were taking the ethanol extract. It can be concluded that the extract does not affect renal or hepatic functions.

One volunteer on initial assessment had elevated levels of glucose, gamma GT, GPT and GOT, which remained elevated at 3, 6, 12 and 18 weeks. Ghloride content was decreased at 3, 6, 12 and 18 weeks. The volunteer was consulted and advised to see

her doctor. The diagnosis was diabetes and recurrence of sarcoidosis. This person was lost to further follow-up.

One volunteer with gout on initial assessment had elevated levels of urea, creatinine and urate, indicating renal dysfunction. These had returned to the normal range at 3 weeks and remained within the normal range at 6 weeks and 12 weeks. This person was lost to further follow-up.

10 Interpretation

In relation to the data shown in Table 17, fasting (8 hours) blood biochemistry and haematology results of seven volunteers who have been taking the ethanol extract for 3 months after the end of the study.

5. Conclusions

The results satisfy the statistical arguments, demonstrating that the ethanol extract reduces pain and body areas experiencing pain for people with rheumatic/arthritis pain.

Although pain relief may occur for some users of the ethanol extract within the first 3-6 weeks of use, the results indicate that pain relief is dependent on the level of pain and the duration of use of the ethanol extract. Thus 12 weeks use of the ethanol extract gives the most efficacious results. Thus from the parametric analysis, the following may be derived.

12 WEEKS USE	
Usual Pain	mean pain level reduced by 52% range of pain reduction 11% - 100%
Usual Pain	residual pain (absence of pain not considered) mean level reduced by 45% range of residual pain reduction 11% - 90%

Present Pain	mean level reduced by 68% range of pain reduction 10% - 100%
Present Pain	residual pain (absence of pain not considered) mean level reduced by 45% range of residual pain 10% - 90%

5 Thus since there is no significant difference
between the significantly reduced mean values of usual
pain and present pain at 12 weeks, it can be stated
that the ethanol extract reduces pain on average
between 45% - 68% within a range of 10% - 100%. The
10 results also demonstrate that the ethanol extract gives
sustained pain relief since there was no breakthrough
pain while taking the ethanol extract. This is further
demonstrable by the return of pain on cessation of use
of the extract and reduction of pain on return to use
of the extract.

15 The pattern of response and the correlation
analysis demonstrates that further pain relief would be
obtained, for those volunteers with residual pain, if
they continue to take the ethanol extract after 12
weeks. Extrapolation indicates a total duration of 22-
20 24 weeks would be required.

The ethanol extract does not appear to have
any affect on renal and hepatic functions or general
blood biochemistry. Similarly there is no effect of
the ethanol extract on haematological parameters.

REFERENCES

1. Beeson PBN, McDermott W and Wyngaarden JB. (1975).
In: Cecil Textbook of Medicine. 5th Edition. Publ.
W B Saunders Co., Philadelphia, London, Toronto.
- 5 2. Webb J and Nash PT. (1990). Polyarthrititis - A
diagnostic approach. Australian Family Physician
19(10):1533-1542.
- 10 3. Kremer JM. (1990). Severe rheumatoid arthritis:
Current options in drug therapy. Geriatrics
45(12):43-48.
- 15 4. Van der Heijde MFM, Van't Hof MA, Van Riel PLCM,
Theunisse LAM, Lubberts EW, Van Leeuwen MA, Van
Rijswijk MH and Van de Patte LBA. (1990). Judging
disease activity in clinical practice in rheumatoid
arthritis: first step in the development of a
disease activity score. Annals. Rheumatic Diseases
20 49:916-920.
- 25 5. Farge D, Liote F, Guillman R and Carpentier A.
(1990). Hyperuricemia and gouty arthritis in heart
transplant recipients. Amer J Med 88:553.
6. Bomaiaske JS, Williamson PK and Zurier RB. (1983).
Prostaglandins and the inflammatory response. Clin
Lab Med 3:695.
- 30 7. Robinson DF, McGuire MB and Levine L. (1974).
Prostaglandins in the rheumatic diseases. Ann N.Y.
Acad Sci 256:318.
- 35 8. Samealsson B, Dahlem SE, Lindgren JA, Rouzer CA and
Serhan CN. (1987). Leukotriene and lipoxins:
structure and biosynthesis and biological effects.
Science 237:1171.

9. Yoneda T and Mundy GR. Monocytes regulate osteoclast activating factor production by releasing prostaglandins. *J Exp Med* 150:338.
- 5 10. Hasselbacher P and Schumacker HR. (1978). Immunoglobulins in trophi and on the surface of monosodium urate crystals. *Arthritis Rheum* 21:353.
- 10 11. Hasselbacher P. (1979). Binding of IgG and complement protein by monosodium urate crystals and other crystals. *J Lab Clin Med* 94:532.
- 15 12. Wigley FM, Fine IT and Newcombe DS. (1983). The role of the human synovial fibroblast in monosodium urate crystal induced synovitis. *J Rheumatol* 10:602.
- 20 13. Dayer JM, Krane SM, Russell RGG and Robinson DF. (1979). Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. *Proc Natl Acad Sci USA* 73:94.
- 25 14. McMillian RM, Vater CA, Hasselbacher P, Hahn J and Harris EH. (1981). Induction of collagenase and protaglandin synthesis in synovial fibroblasts treated with monosodium urate crystals. *J Pharm Pharmacol* 33:382.
- 30 15. Rae SE, Davidson EM and Smith JH. (1982). Leukotriene B4 - An inflammatory mediator in gout. *Lancet* 2:1122.
- 35 16. Bomalaski JS, Baker DG, Brophy LM and Clark MA. (1990). Monosodium urate crystals stimulate phospholipase A2 enzyme activities and the synthesis of a phospholipase A2-activating protein. *The J Immunol* 145:3391-3397.

17. Roubenoff R. (1990). Gout and hyperuricemia. Rheumatic Disease Clinics of North America 16(3):539-551.
- 5 18. Leventhal LJ, Levin RW and Bomalaski JS. (1990). Peripheral arthrocentesis in the workup of acute low back pain. Ach Phy Med Rehabil 71:253-254.
- 10 19. Hess B and Binswanger U. (1990). Acute uric acid nephropathy in two gouty patients with moderate hyperuricemia and high urine acidity. Klin, Wochenschr 68:874-879.
- 15 20. Kirwan JR, de Saintonge CDM, Joyce CRB and Currey HLF. (1983). Clinical judgment in rheumatoid arthritis I. Rheumatologists opinion and the development of paper patients. Ann Rheum Dis 42:644-647.
- 20 21. Kirwan (1983). Clinical judgment in rheumatoid arthritis II. Judging current disease activity in clinical practice. Ann Rheum Dis 42:648-651.
- 25 22. Kirwan JR, de Saintonge CDM, Joyce CRB and Currey HLF. (1986). Inability of rheumatologists to describe their true policies for assessing rheumatoid arthritis. Ann Rheum Dis 45:156-161.
- 30 23. Sany, Clinical and Experimental Rheumatology 8 (Suppl. 5) 81-88 (1990).
24. Kumar et al., JAP1 (1990) Vol 38 No. 6 400-402.
- 35 25. Foreman et al., (1990). Child Nephrol Urol 10:115-118.
26. Curtin Microbiological Sciences 1 No. 5 (1984).

27. Webb *et al.* Australian Family Physician 19 No. 10
October 1990, 1533-1542.
28. British Pharmacopoeia (1934).
- 5 29. Kohli *et al.*, Oct 1967. Indian Jour Med Res
55:1099-1102.
- 10 30. Kulshrestha *et al.*, 1 Jan 1970. Indian Jour Med Res
58:99-102.
31. European Pharmacopoeia Standards.
- 15 32. McLeod *et al.*, (1988). Phytochemistry 27 No. 2,
373-375.
33. Salzer CRC Critical Reviews in Food Science and
Nutrition Nov 1977.
- 20 34. Lewis *et al.*, (1985). Int J Crude Drug Res 3 No. 1,
27-32.
35. Al-Hindawi *et al.*, (1989). J Ethnopharmacology
26:163-168.
- 25 36. Price *et al.*, (1987). Critical Reviews in Food
Science and Nutrition 26 No. 1.
- 30 37. In.. Laboratory Techniques in biochemistry and
molecular biology. Applications of HPLC in
Biochemistry by A. Fallon, R F G Booth and L D
Bell. Publ. Elsevier Amsterdam: N.Y. Oxford. Vol.
17. 1990.

TABLE 1

1. No medication	9
2. Vitamins	1
3. Analgesics	3 (BP or Panadol)
4. Hormones	1 (Oestrogen)
5. Anti-inflammatory	1 (Inderel)

TABLE 2

TIME IN WEEKS					
Present Pain Intensity	0	3	6	12	Σ
EX = Excruciating	0	0	0	0	0
HOR = Horrible	1	1	0	1	3
DIST = Distressing	1	2	1	1	5
DC = Discomforting	7	2	6	1	16
M = Mild	4	8	3	8	23

$$r = -0.972 \quad p > 0.001 < 0.01$$

TABLE 3

Weeks	Σ Usual Pain	χ^2	P	Σ Present Pain	χ^2	P
0	496.4			298.7		
3	354.2	40.7	<0.001	212.8	24.7	<0.001
6	252.2	29.4	<0.001	189.6	2.5	>0.1
12	261.5	0.34	>0.5	125	33.4	<0.001

$$r = 0.917 \quad p > 0.02 < 0.05$$

TABLE 4

McGILL PAIN RATING INDEX						
Usual Pain Control 1 \pm 0.33				Present Pain Control 1 \pm 0.36		
Case	3 weeks	6 weeks	12 weeks	3 weeks	6 weeks	12 weeks
1	0.38	0.60	0.68	0.47	0.10	0.56
2	2.27	0.57	0	2.27	0.57	0
3	0.53	0.15	0.10	0.11	0	0
4	0	0.26	0.46	0	0.26	0.46
5	0.30	0.08	0	0.10	0	0
6	0.60	0.40	0.28	0.14	0.40	0
7	0.87	0.36	0.62	1.3	0.57	-
8	1.00	0.79	0.60	0	0.15	0
9	0.77	0.81	0.57	0.57	0.82	0.18
10	0.82	0.78	0.81	0.84	0.82	0.86
11	0.42	0.44	0.20	0.62	0	0.10
12	1.02	0.86	0.89	1.0	0.91	0.90
13	1.10	0.94	0.82	1.1	1.10	0.85
14	0.95	0.78	0.79	0.36	0.80	0.52
15	0.28	0.19	0.33	0	0.06	0
X *	0.76	0.53	0.48	0.59	0.43	0.32
\pm sd	\pm 0.51	\pm 0.28	\pm 0.30	\pm 0.61	\pm 0.37	\pm 0.34
X **	0.53		0.55	0.40	0.50	0.55
\pm sd	\pm 0.28		\pm 0.25	\pm 0.25	\pm 0.30	\pm 0.29

* X \pm sd = total observations** X \pm sd = total observations minus observations > 1 and zero

TABLE 5

Usual Pain Index	3 Weeks		6 Weeks		12 weeks	
	t'	P	t'	P	t'	P
X \pm sd *	1.53	>0.05 <0.1	4.20	<0.001	4.52	<0.001
X \pm sd **	3.82	<0.001	-	-	4.10	<0.001
Present Pain Index						
X \pm sd *	2.24	>0.02 <0.05	4.28	<0.001	5.23	<0.001
X \pm sd **	3.46	>0.001 <0.01	3.85	<0.01	3.21	>0.001 <0.01

TABLE 6

Weeks	Usual Body Areas	t'	P	Present Body Areas	t'	P
0	3 \pm 1.91 n = 77			2.15 \pm 1.2 n = 68		
3	1.9 \pm 1.1 n = 53	4.2	<0.001	1.48 \pm 0.63 n = 27	3.5	<0.001
6	1.59 \pm 0.81 n = 45	5.6	<0.001	1.26 \pm 0.64 n = 27	4.7	<0.001
12	1.7 \pm 0.77 n = 45	5.4	<0.001	1.27 \pm 0.56 n = 33	5.0	<0.001

TABLE 7

Weeks	Mean Body Areas	Sum Total Pain Index
	Usual	Usual
0	3	496.4
3	1.9	354.2
6	1.59	252.2
12	1.7	261.5

$$r = 0.973 \quad p > 0.001 < 0.01$$

TABLE 8

Weeks	Mean Body Areas	Sum Total Pain Index
	Present	Present
0	2.15	298.7
3	1.48	212.2
6	1.26	189.6
12	1.27	125.0

$$r = 0.915 \quad p > 0.002 < 0.05$$

TABLE 9

Present Original Present Pain %	Ratio Days Development of Pain/Restarting the Ethanol Extract Ratio
60	42/49 = 0.86
100	14/28 = 0.50
51	13/15 = 0.87
95	21/36 = 0.58
39	29/36 = 0.80
100	7/46 = 0.15

$$r = 0.827 \quad p > 0.02 < 0.05$$

TABLE 10

McGILL PAIN RATING INDEX				
	Time in Weeks			
	Week 12	Week 0	Week 0 χ^2	Week 0 P
Usual Pain	99.3	137.5	14.7	<0.001
Present Pain	36.6	116.3	186.0	<0.001
Usual Body Areas	34	47	4.97	>0.02 <0.05
Present Body Areas	11	40	76.4	<0.001

TABLE 11

McGILL PAIN RATING INDEX							
Pain	Wk 0	Wk 3	Wk 6	Wk 3	Wk 6	Wk 3	Wk 6
Usual	137.5	85.3	60.7	19.8	7.1	<0.001	<0.001
Present	116.3	39.2	25.6	51.1	4.7	<0.001	>0.02 <0.05
UBA	47	22	19	13.3	0.4	<0.001	0.5
PBA	40	5	13	30.6	12.8	<0.001	<0.001

UBA = usual body areas total number
PBA = present body areas total number

TABLE 12

Time in Weeks (Wks)	Usual Pain (UP)
0	262.4
3	130.1
6	97.2
12	99.3
0	137.5
3	85.3
6	52.6

TABLE 13

Time in Weeks (Wks)	Present Pain (PP)
0	144.6
3	83.2
6	26.5
12	36.6
0	110.0
3	29.2
6	13.7

TABLE 14

Time in Weeks (Wks)	Usual Body Areas (UBA)
0	109
3	31
6	24
12	34
0	47
3	20
6	16

TABLE 15

Time in Weeks (Wks)	Present Body Areas (PBA)
0	48
3	24
6	7
12	11
0	40
3	6
6	13

TABLE 16

BLOOD BIOCHEMISTRY			
Phosphate	Normal	Sodium	Normal
Total protein	Normal	Chloride	Normal
Albumin	Normal	Bicarbonate	Normal
Globulins	Normal	Glucose	Normal
Cholesterol	Normal	Total bilirubin	Normal
Triglycerides	Normal	Conjugated bilirubin	Normal
Urea	Normal	Gamma GT	Normal
Creatinine	Normal	GPT	Normal
Urate	Normal	GOT	Normal

TABLE 17

BLOOD BIOCHEMISTRY			
Sodium	Normal	ALT (SGPT)	Normal
Potassium	Normal	AST (SGOT)	Normal
Chloride	Normal	LDH	Normal
Bicarbonate	Normal	Calcium	Normal
Other anions	Normal	Adjusted for Albumin	Normal
Glucose	Normal	Phosphate	Normal
Urea	Normal	Total protein	Normal
Creatinine	Normal	Albumin	Normal
Urate	Normal	Globulins	Normal
Total bilirubin	Normal	Iron	Normal
Conjugated bilirubin	Normal	Cholesterol	Normal
ALK Phos.	Normal	Triglycerides	Normal
Gamma GT	Normal		Normal

CLAIMS

1. A method for the treatment of an inflammatory complaint including the step of administration of a compound of general formulae I to XII described herein in FIG 1 or mixtures thereof to a subject suffering from said inflammatory complaint.
2. A method as claimed in Claim 1 wherein said compounds comprise butylphthalide, sedanenolide and sedanolide in both cis and trans forms.
3. A composition for use in the treatment of an inflammatory complaint comprising one or more compounds of general formulae I to XII described herein in FIG 1 or mixtures thereof also including a pharmaceutically acceptable filler.
4. A composition as claimed in Claim 3 wherein the filler is included in said composition on a basis of 5-95% by weight.
5. A composition as claimed in Claim 4 wherein said filler is included in said composition on a basis of 50% or more by weight.
6. A composition as claimed in Claim 5 wherein said filler is included in said composition on a basis of 75% or more by weight.
7. A composition as claimed in Claim 1 wherein the filler is dicalcium phosphate.
8. A process of obtaining a plant extract useful in treatment of inflammatory complaints including the steps of:
- (i) contacting plant material with an organic solvent which may extract soluble components of the plant material to produce a solvent extract;
 - (ii) separating the mixture of plant material and solvent so as to remove particulate material therefrom;
 - (iii) concentrating the extract by removing the solvent.

- 5 9. A process as claimed in Claim 8 wherein said plant material is crushed plant seed which has an average dimension of 1.5 x 1.55 mm and a thickness of 0.5 mm which may be comminuted to provide finely divided particles of an average diameter of 0.1 - 0.2 mm.
- 10 10. A process as claimed in Claim 9 wherein the comminuted seed is suspended in an organic solvent whereby the ratio of seed to solvent is 1 kg of seed in 5-16 litres of solvent.
11. A process as claimed in Claim 10 wherein the organic solvent is ethanol.
12. A process as claimed in Claim 11 wherein the ethanol has a concentration of 95-98%.
- 15 13. A process as claimed in Claim 11 wherein the ethanol has a concentration of 99.99%.
14. A process as claimed in Claim 10 wherein the comminuted seed is mixed with the solvent under slow agitation until all extractable components in the seed have been fully extracted with the organic solvent.
- 20 15. A process as claimed in Claim 14 wherein the particulate plant material is removed by filtration using filters having a pore size of from 0.2 to 10 microns.
- 25 16. A process as claimed in Claim 15 wherein after removal of particulate plant material the organic solvent extract is subsequently concentrated to remove volatile material leaving a residue of non-volatiles.
- 30 17. A process as claimed in Claim 16 wherein the residue is further concentrated under vacuum so that there is a 50% weight loss due to the removal of organic solvent and water to provide a viscous liquid or concentrate.
- 35 18. A viscous liquid or concentrate prepared from the process of Claim 17 when used as a therapeutic agent.
19. A viscous liquid or concentrate prepared from

ethanolic extraction of celery seed containing butylphthalide, sedanenolide and sedanolide.

5 20. A therapeutic agent used for alleviation of inflammatory complaints including the viscous liquid or concentrate of Claim 19.

21. A method for the treatment of inflammatory complaint including the step of administration of the therapeutic agent of Claim 20.

10 22. An ethanol extract obtained from celery seed which upon HPLC analysis has peaks A, B and C as described herein in relation to Examples I - VI.

1/39

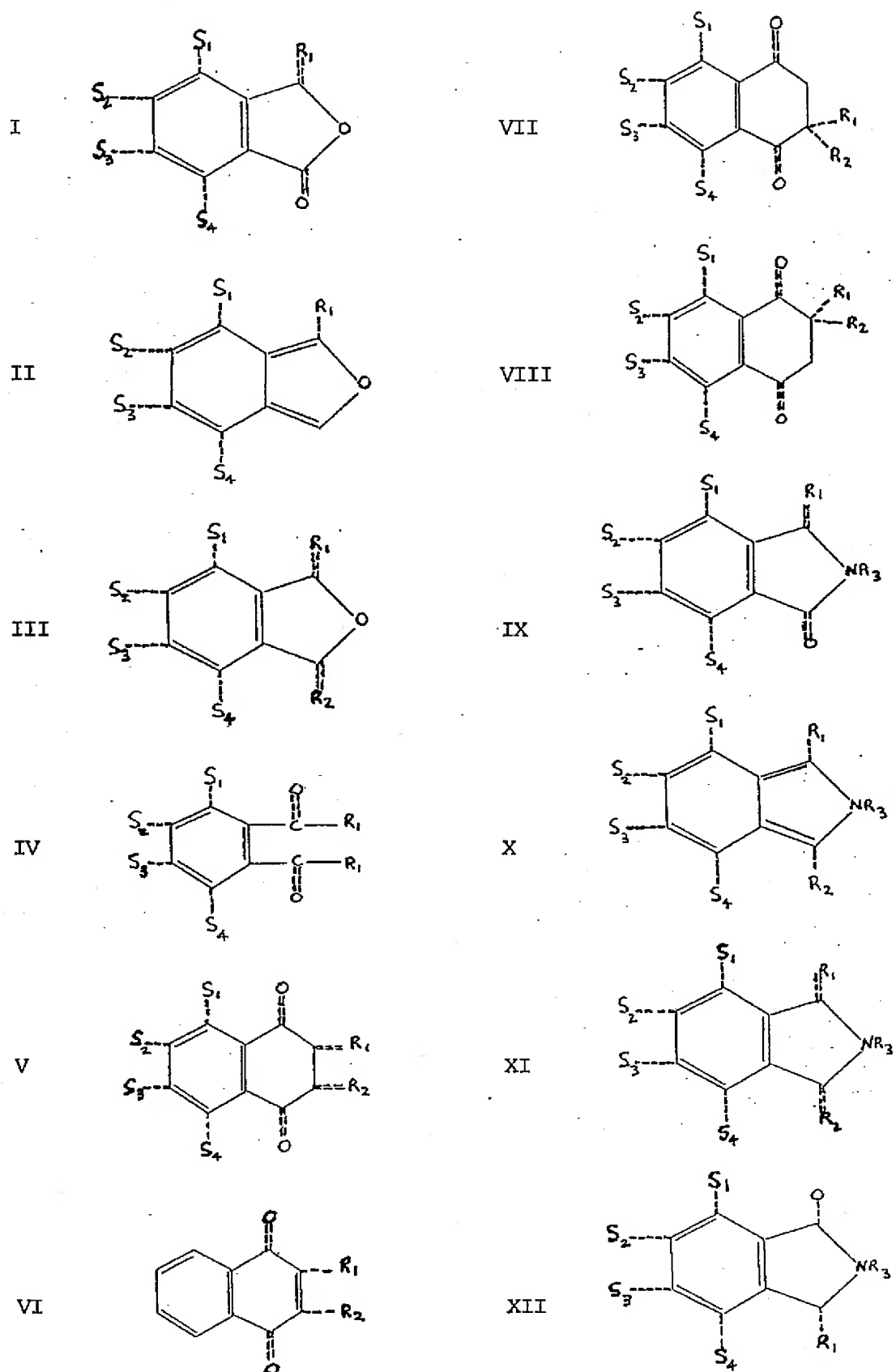


FIG 1

2/39

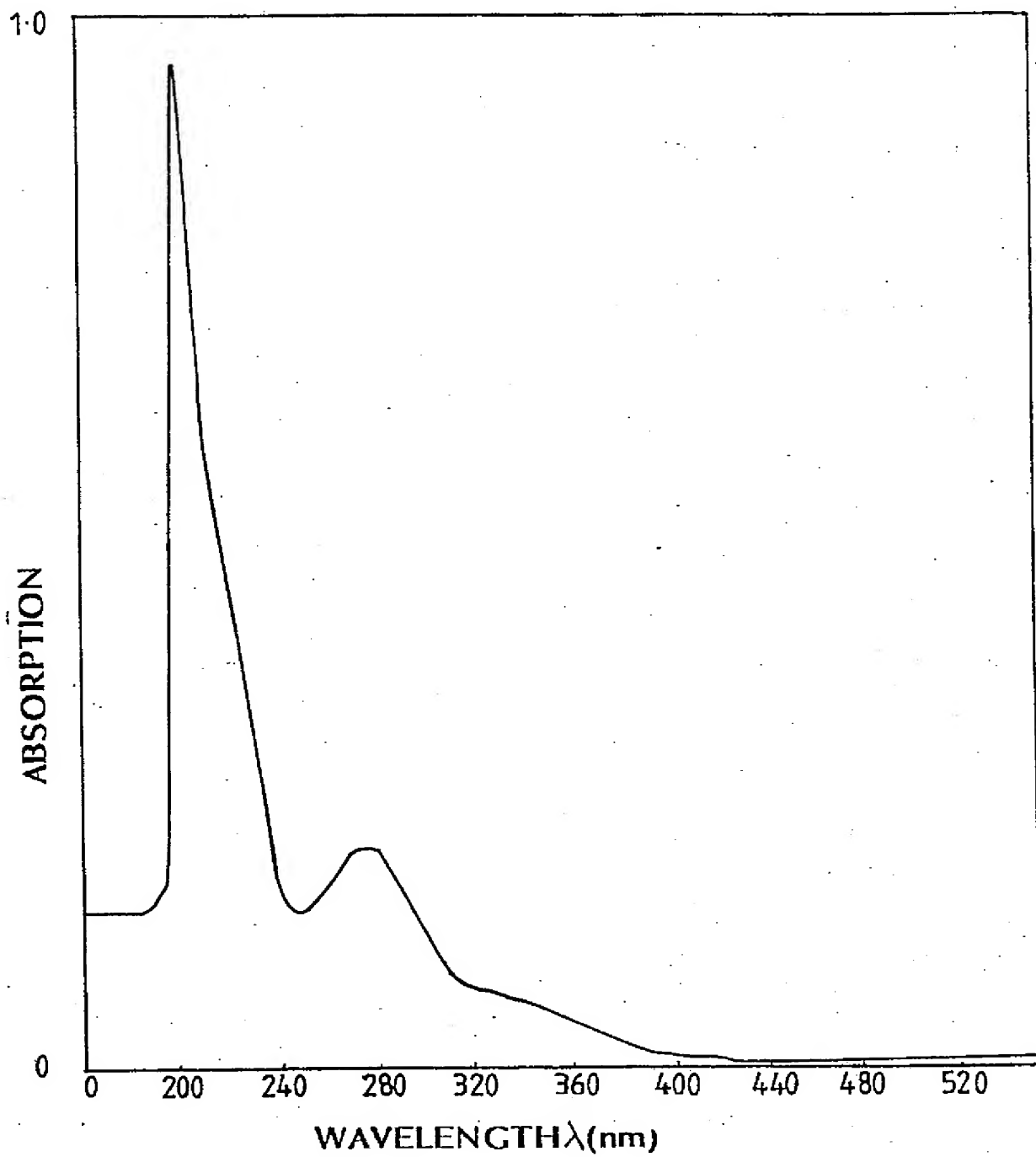


FIG. 2

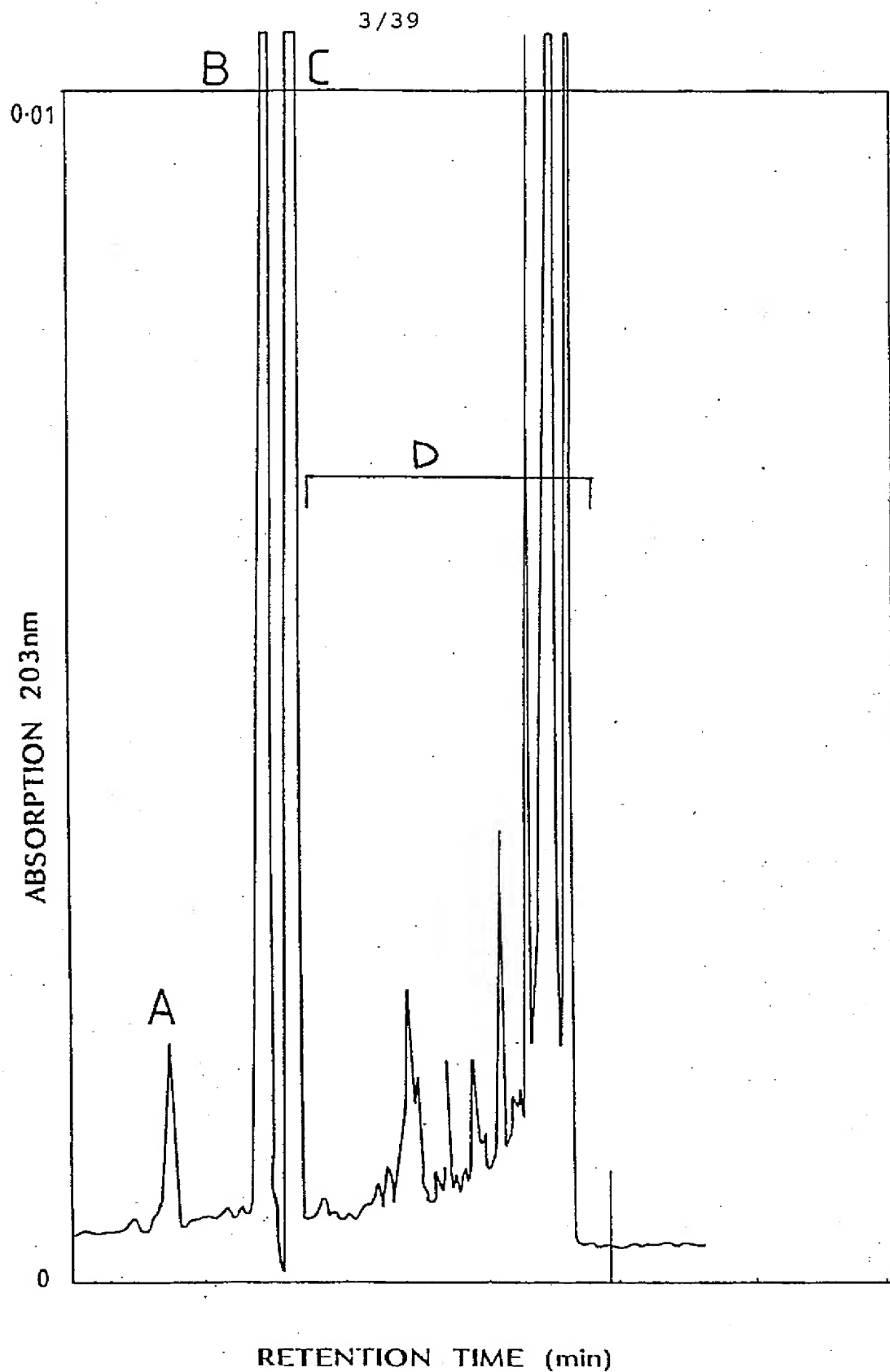


FIG. 3

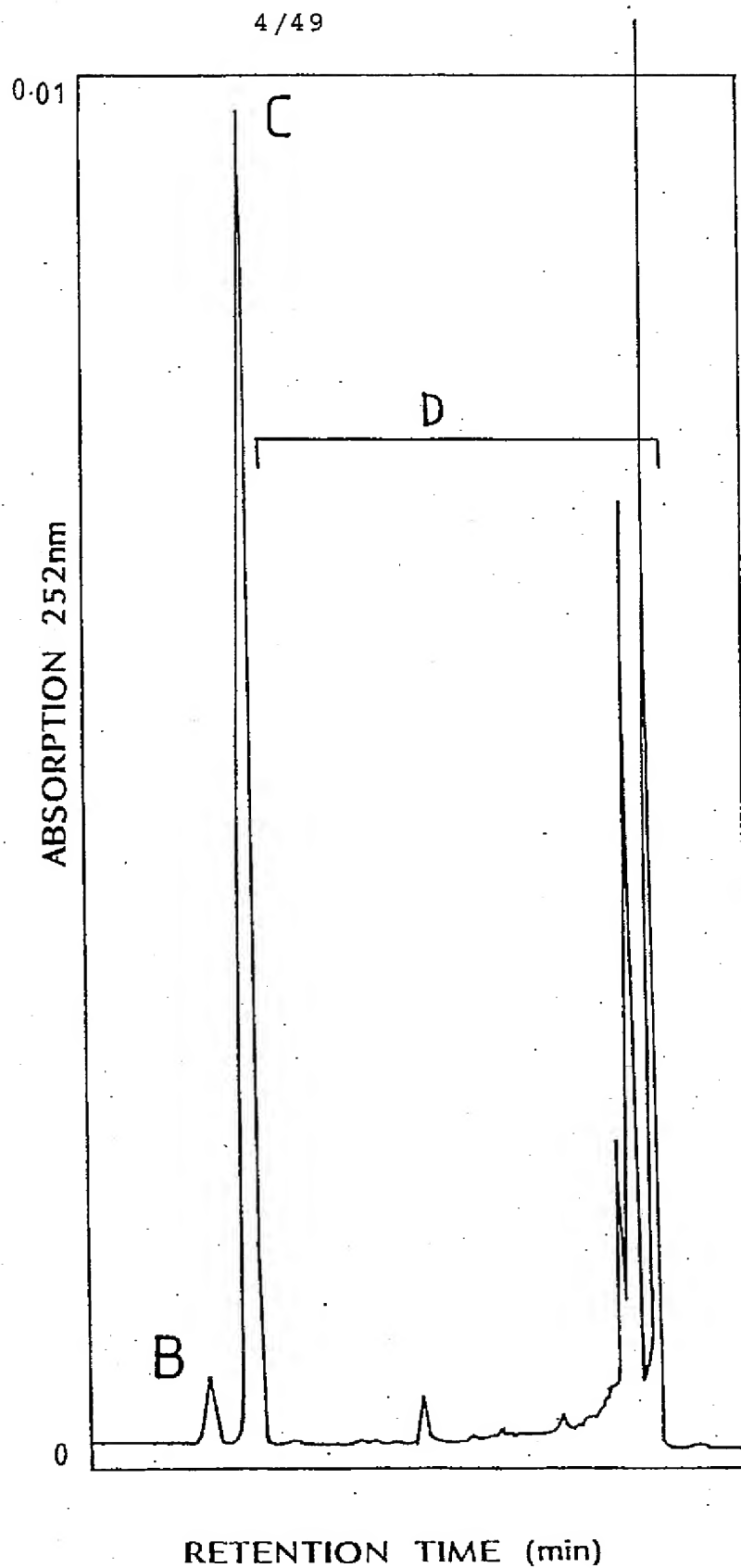


FIG.4

5/39

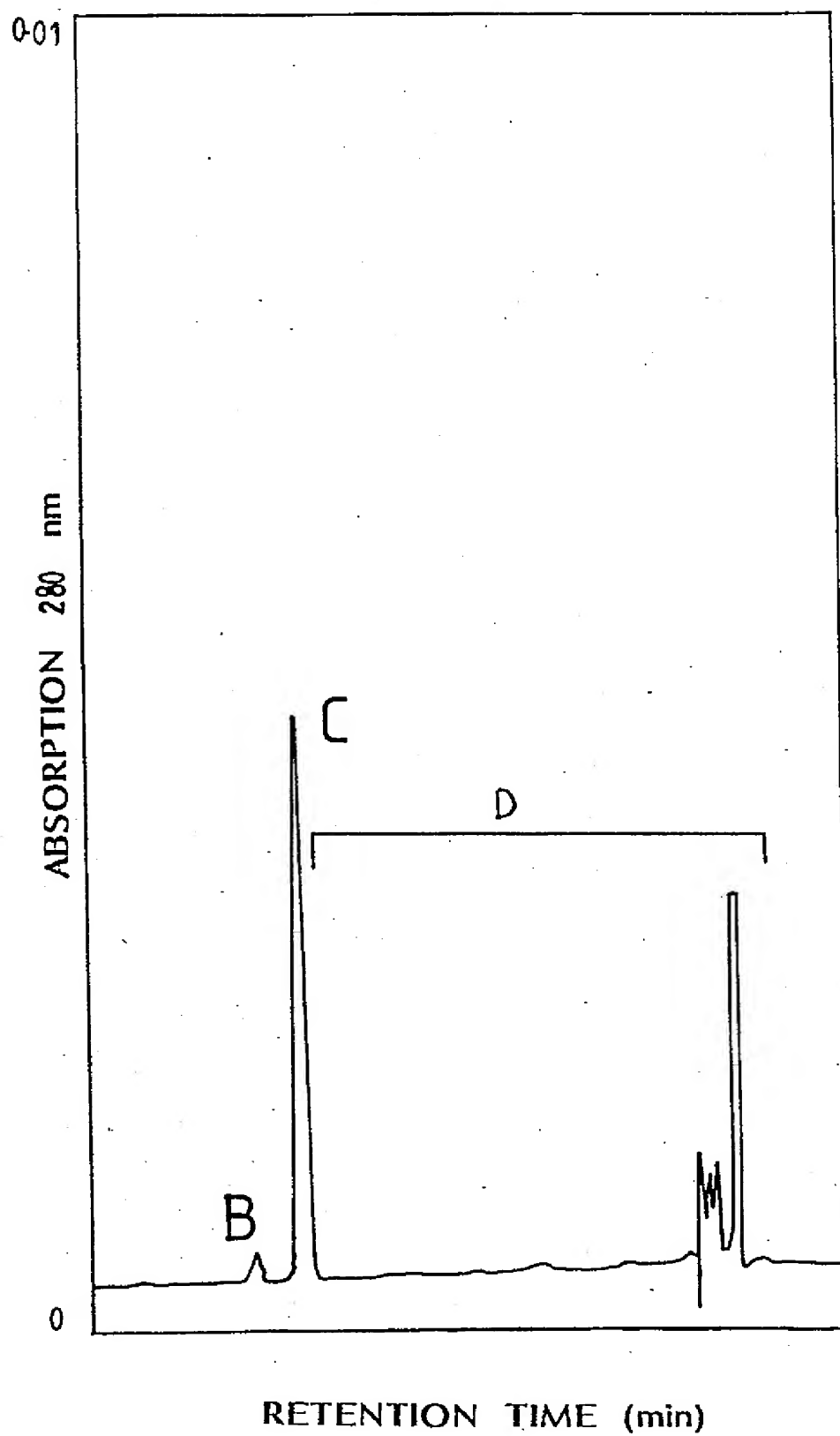
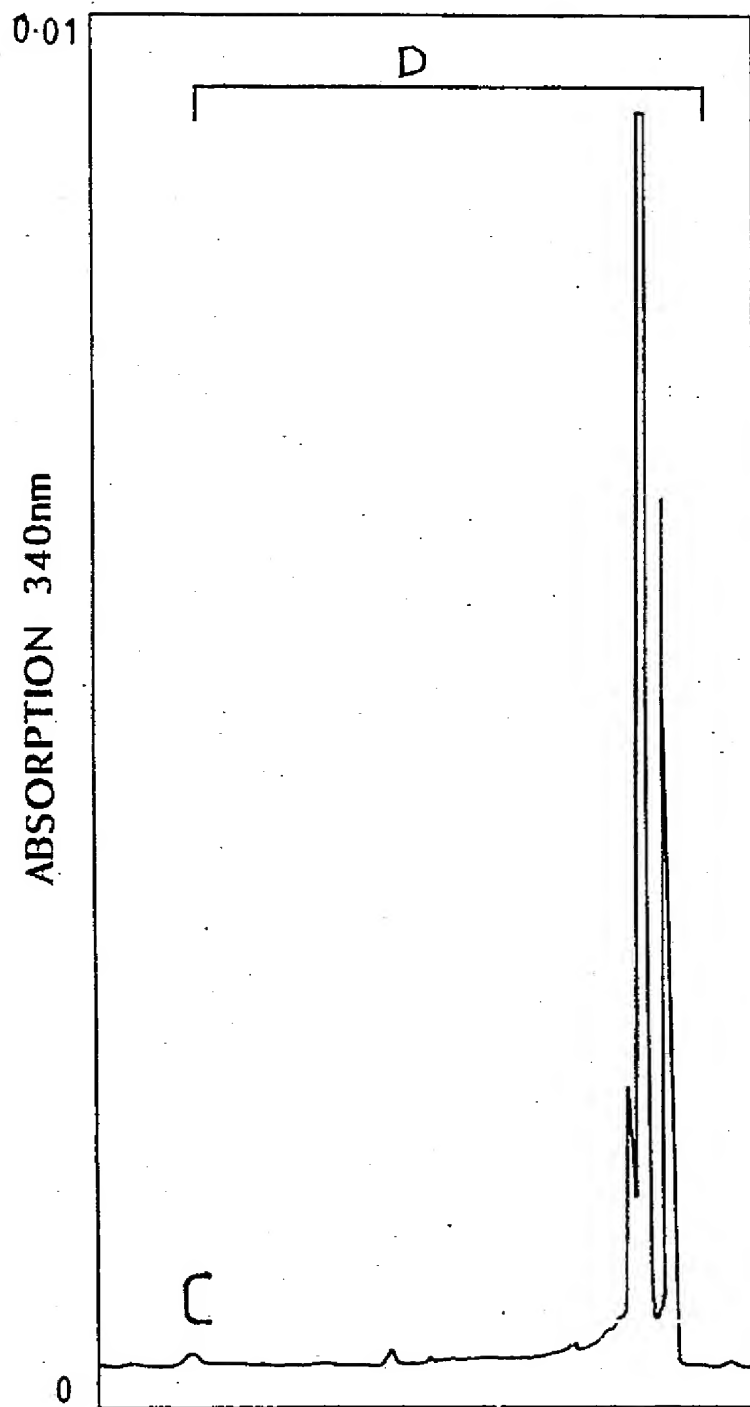


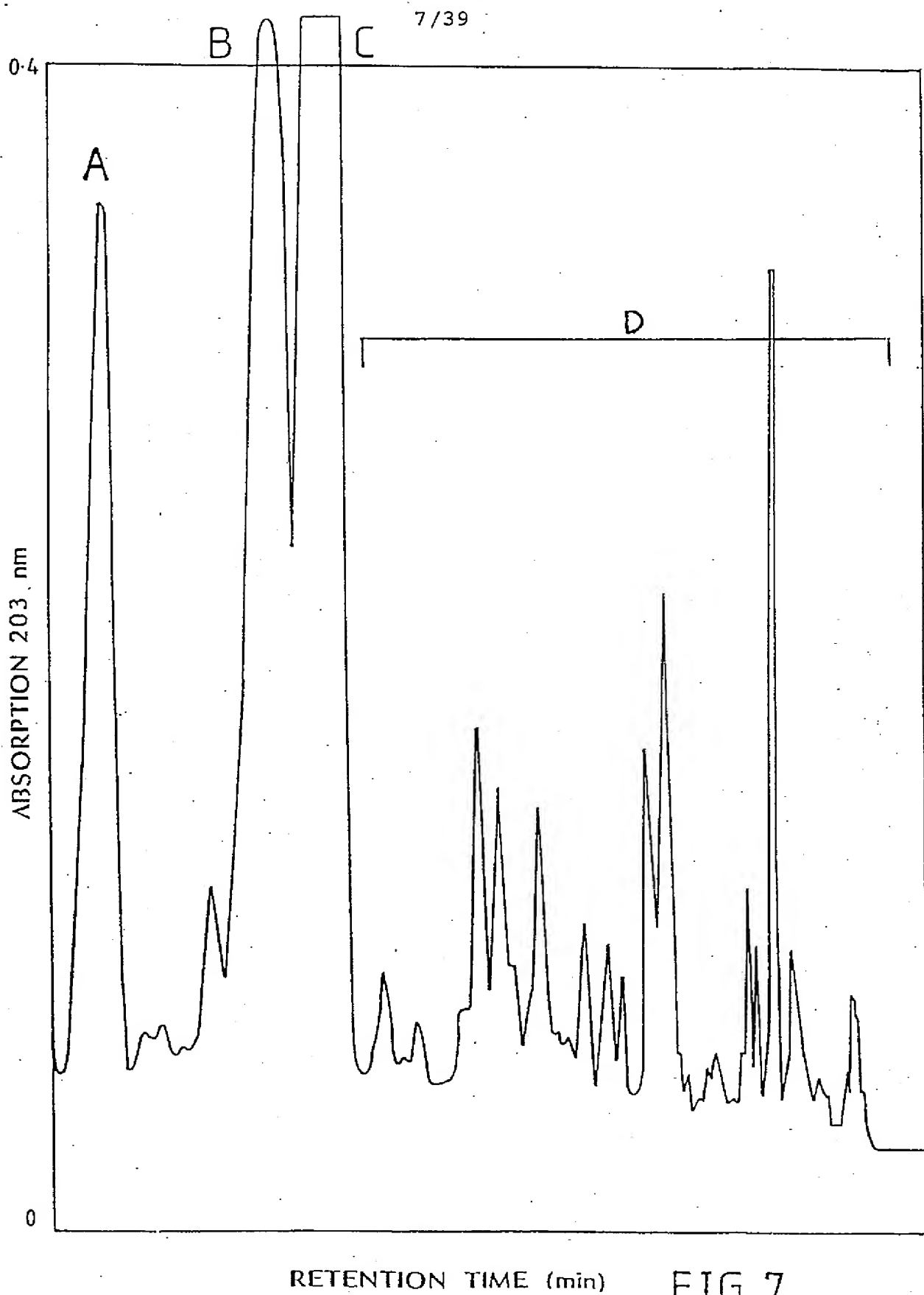
FIG. 5

6/39



RETENTION TIME (min)

FIG. 6



8/39

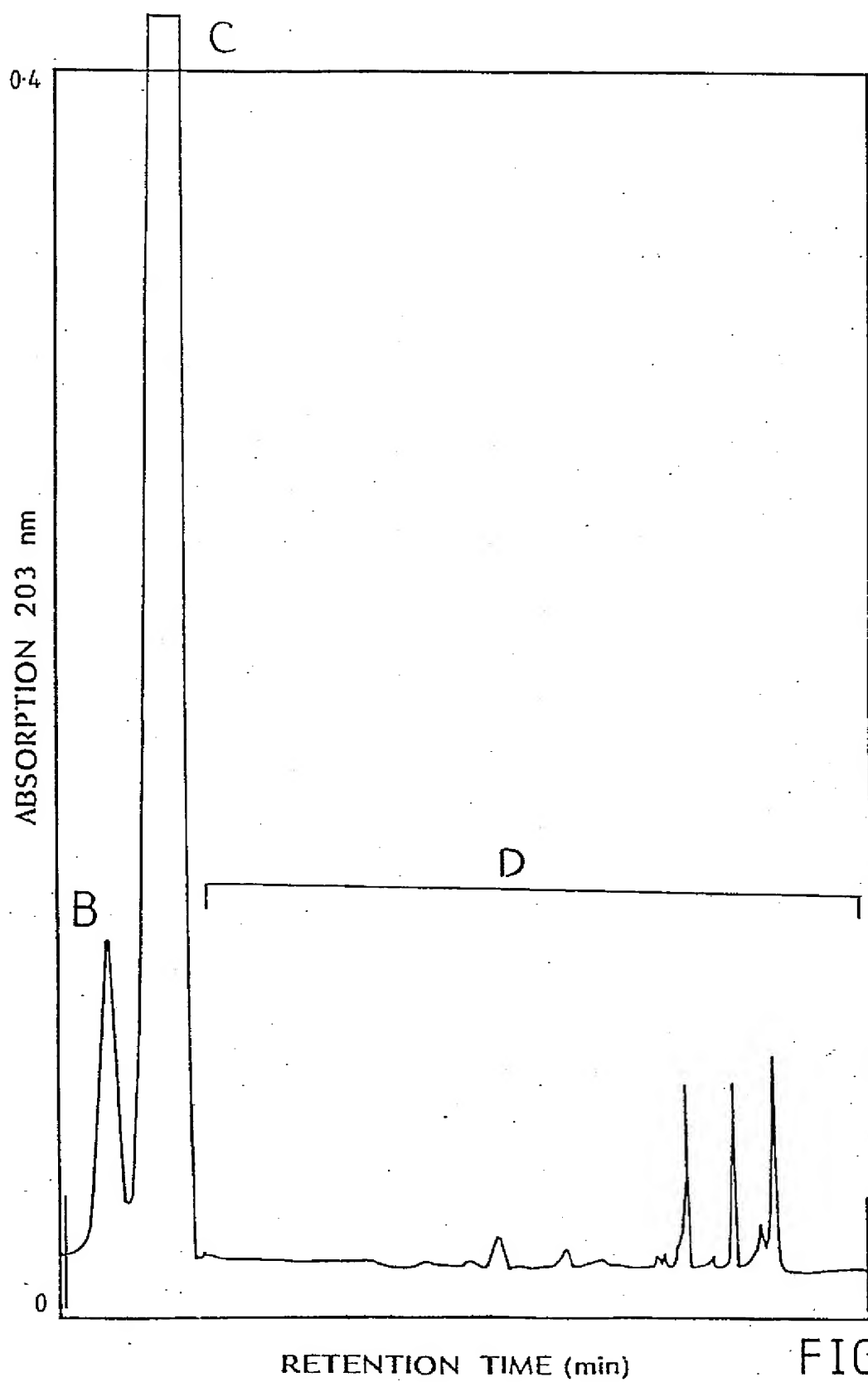
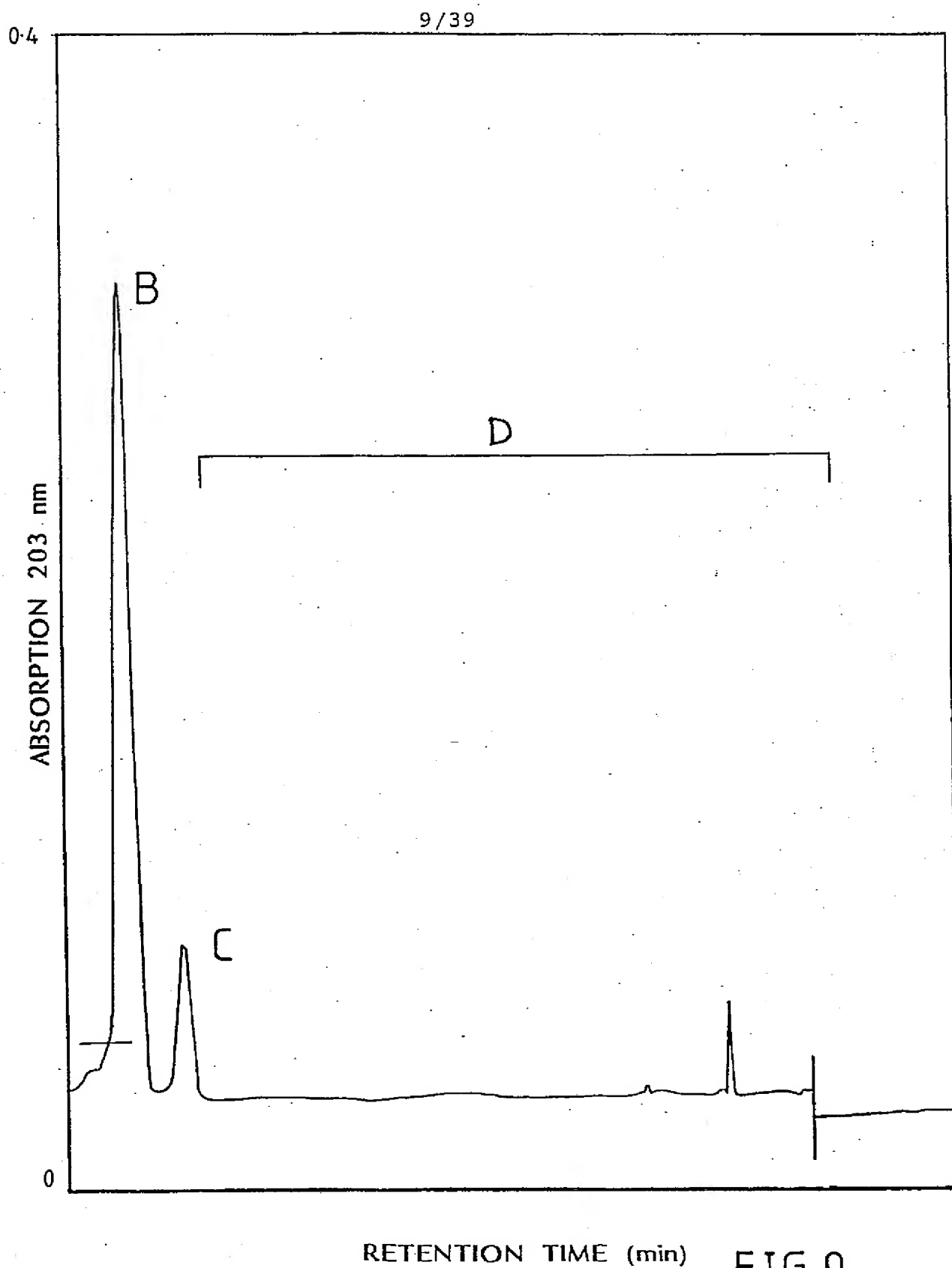


FIG. 8



10/39

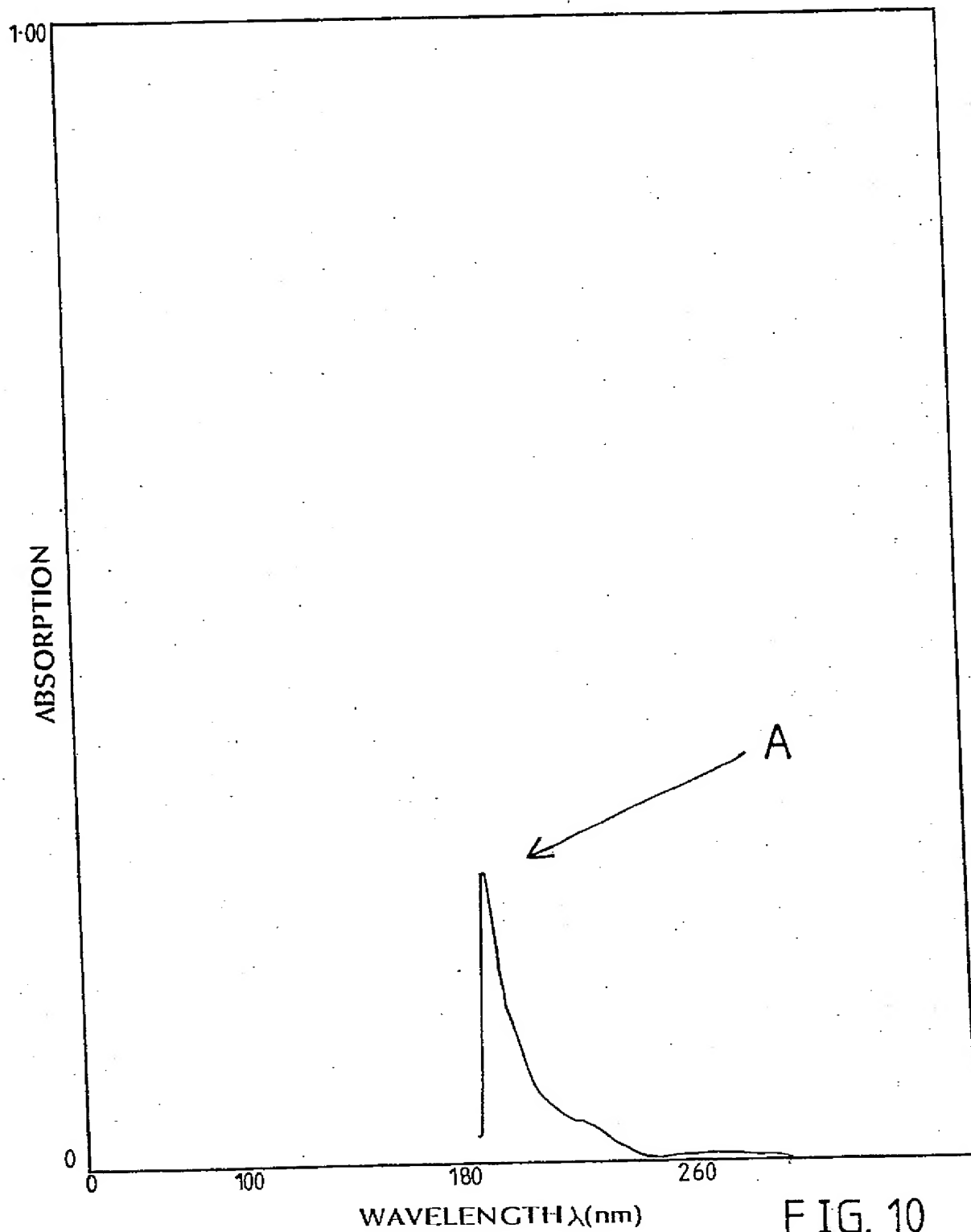


FIG. 10

11/39

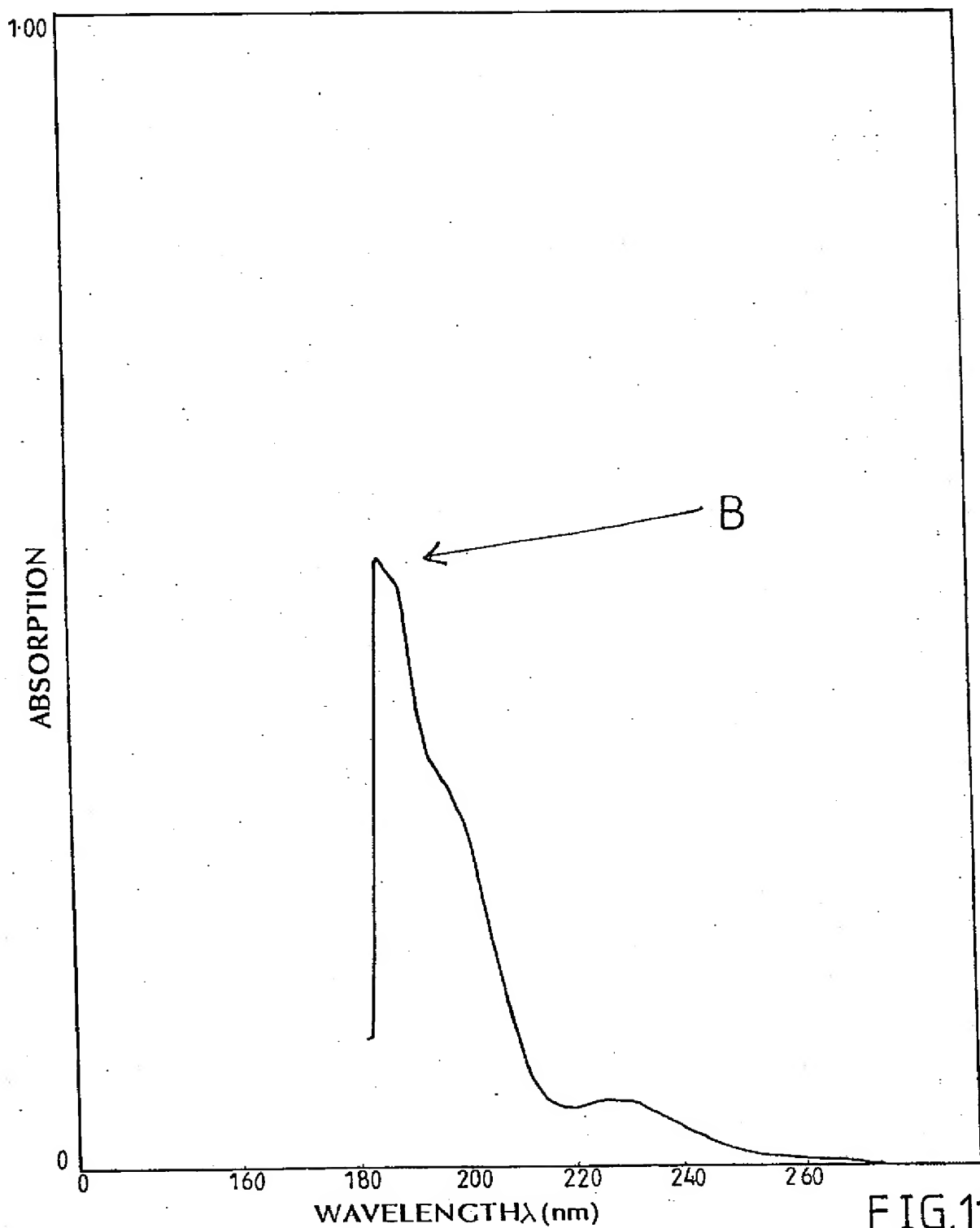


FIG.11

12/39

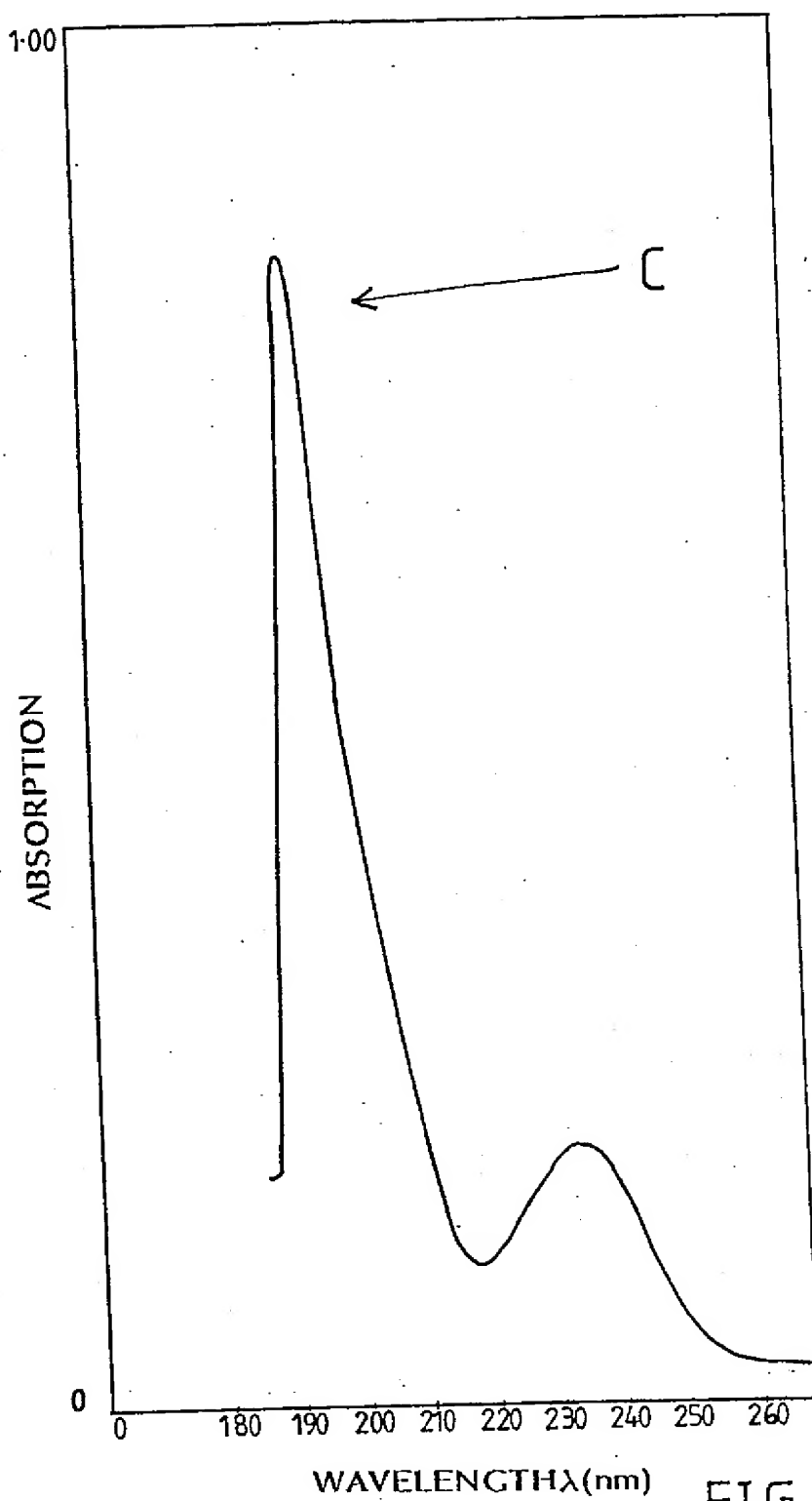


FIG. 12

13/39

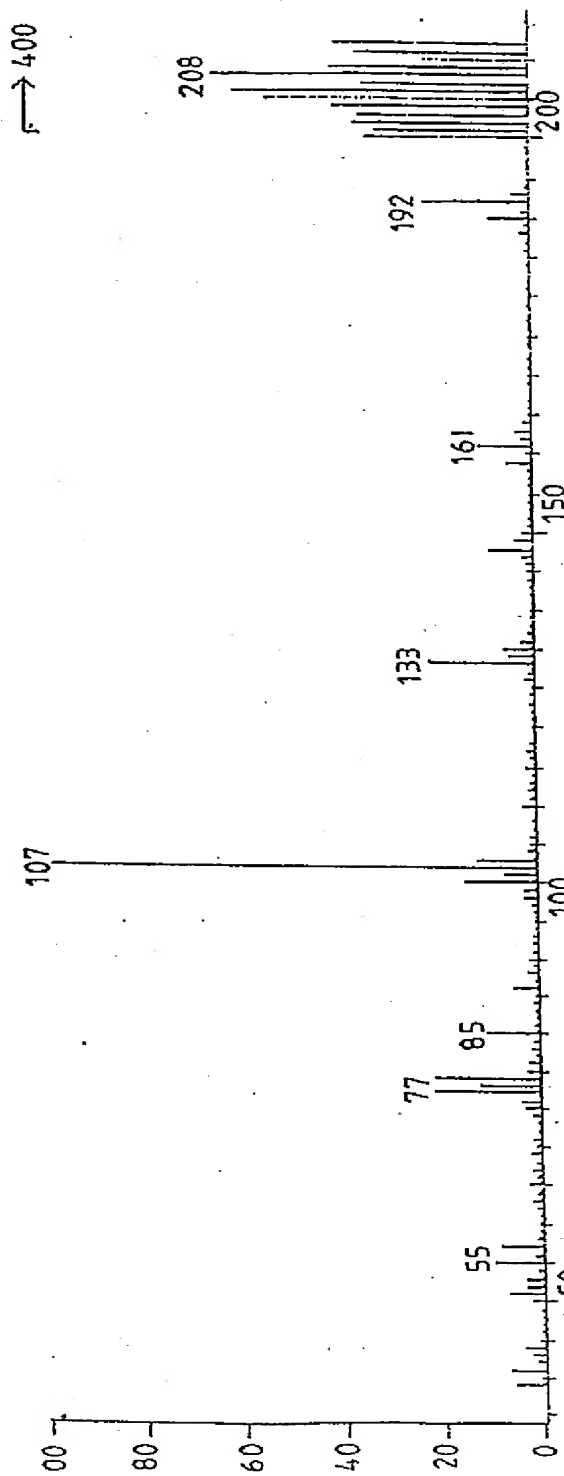


FIG 13A

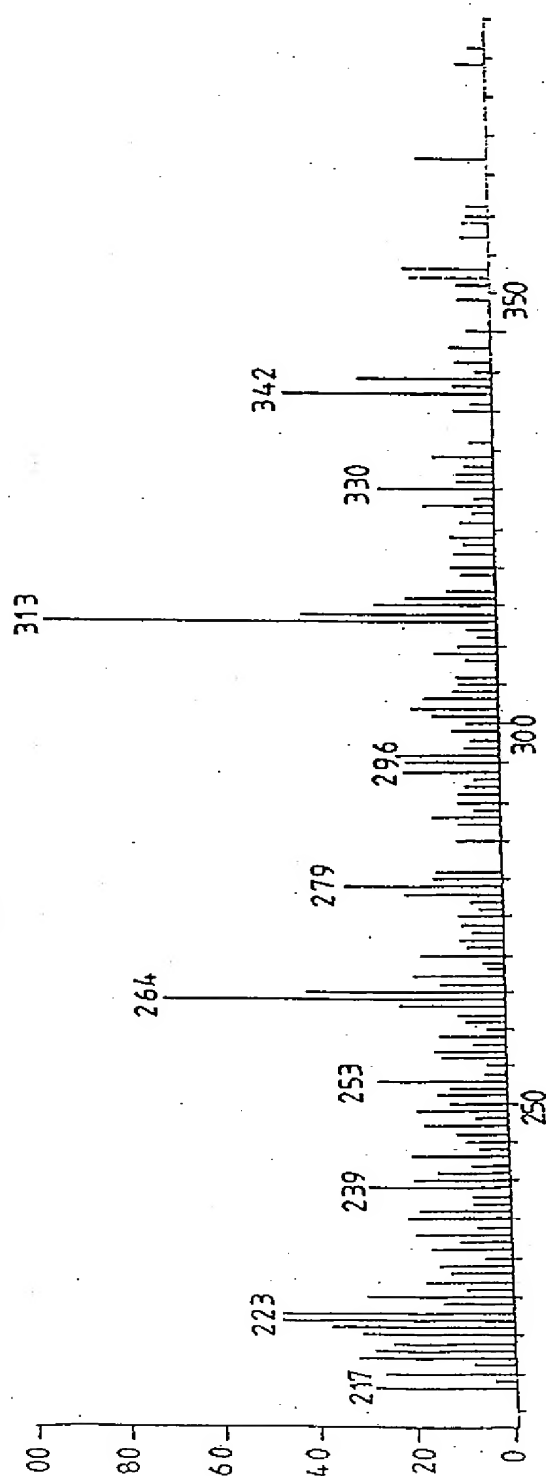


FIG 13B

Sample 3.2

exp2 pulse sequence. stdin

```

SAMPLE          DEC. & VT      HI
date    Apr 13 93   dn          0
solvent  CDCl3     do/         nnn
file     /home/vnm" dn          c
rl/vnmrjys/data/D5" dnm        200
unter_3.2 dmi/         30
ACQUISITION      dpmr         28.0
sfrq 299.949      tempo
tn    HI          PROCESSING
at    3.744      xtlila
ap    29952      proc      ft
aw    4000.0     fn        not used
fb    2200      math      f
bs    18
bpwr  55
bw    7.0      wexp
d1    0        wba
lof    725.8    xnt      DISPLAY
nt     32
ct     32      ap        2.9
alock  n        mp        2712.6
gain   not used ve        200
FLAGS  ac        0
li      n        mc        330
ln      n        hzmm      8.22
dp      y        l5        308.05
ha      nn       rf1        2375.1
                   rfp        2174.6
                   th         20
                   lna        1.000
                   nm cdc ph

```

14/39

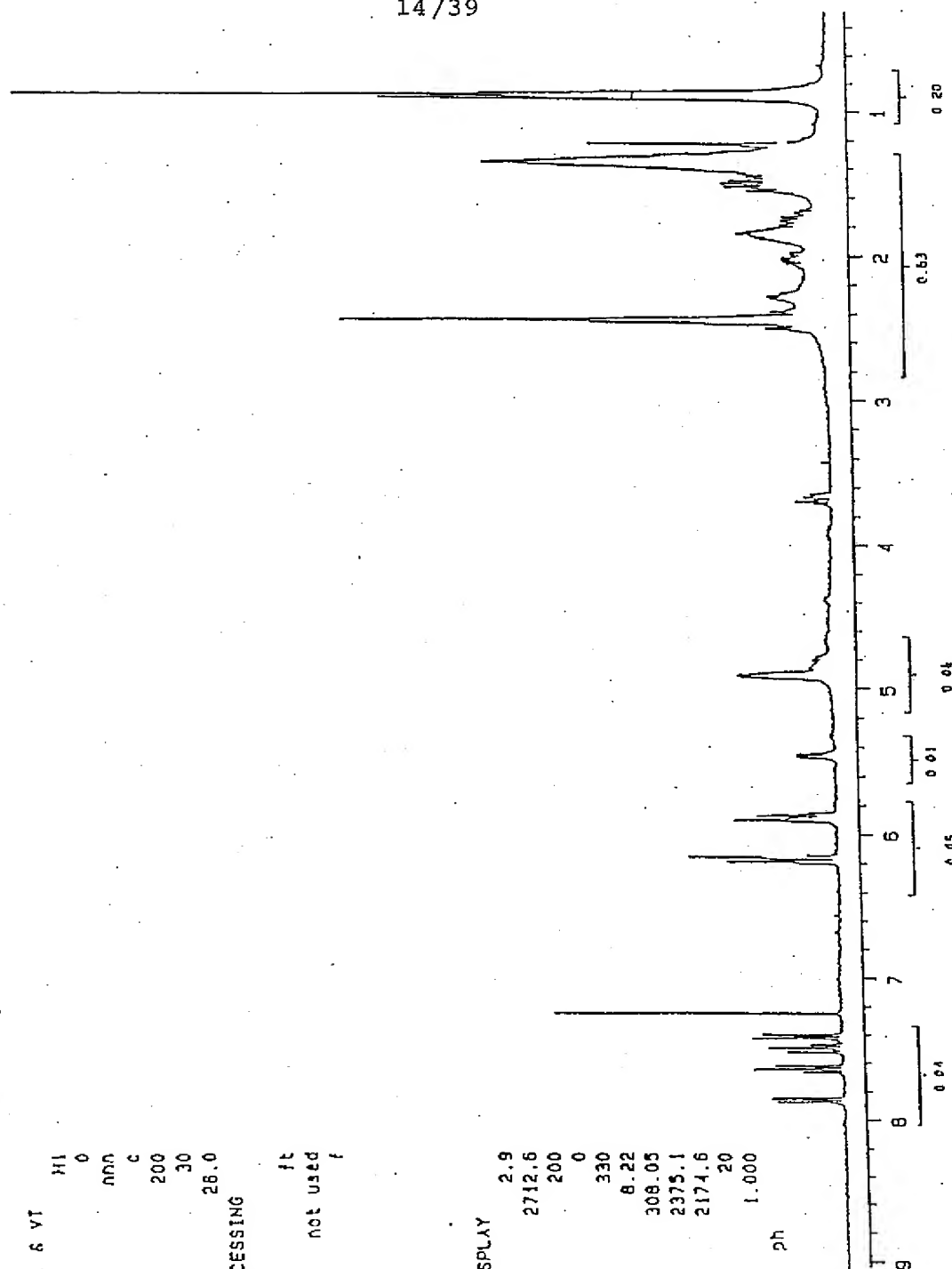


FIG. 14

15/39

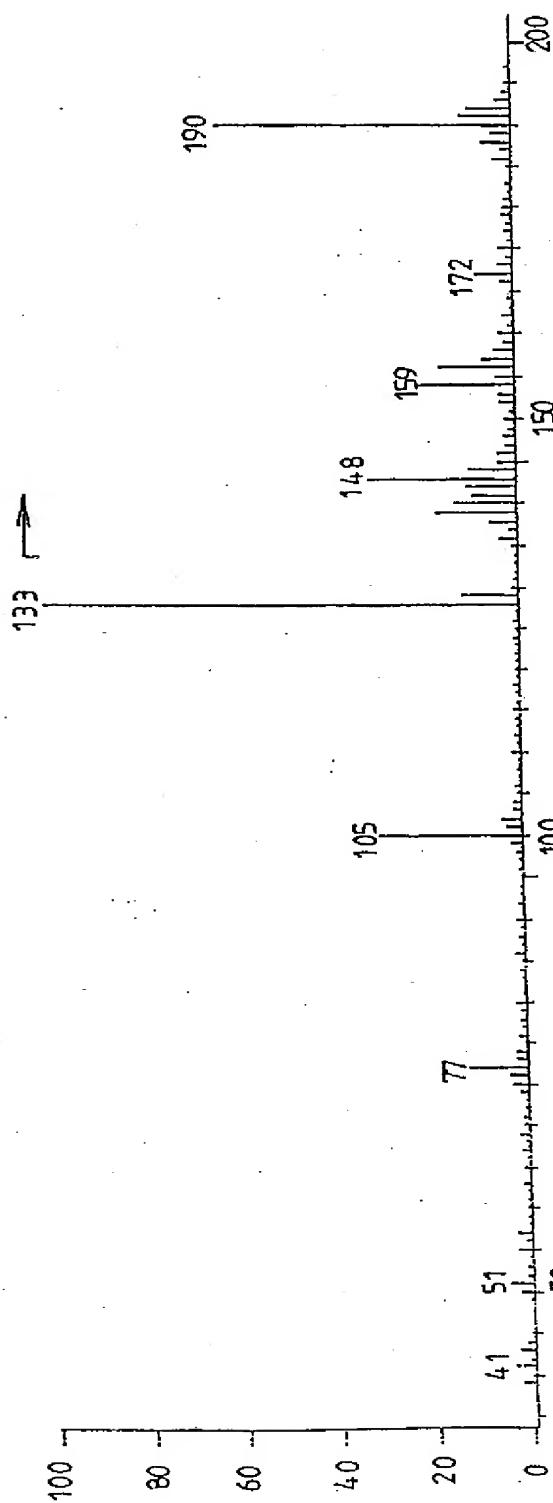


FIG 15A

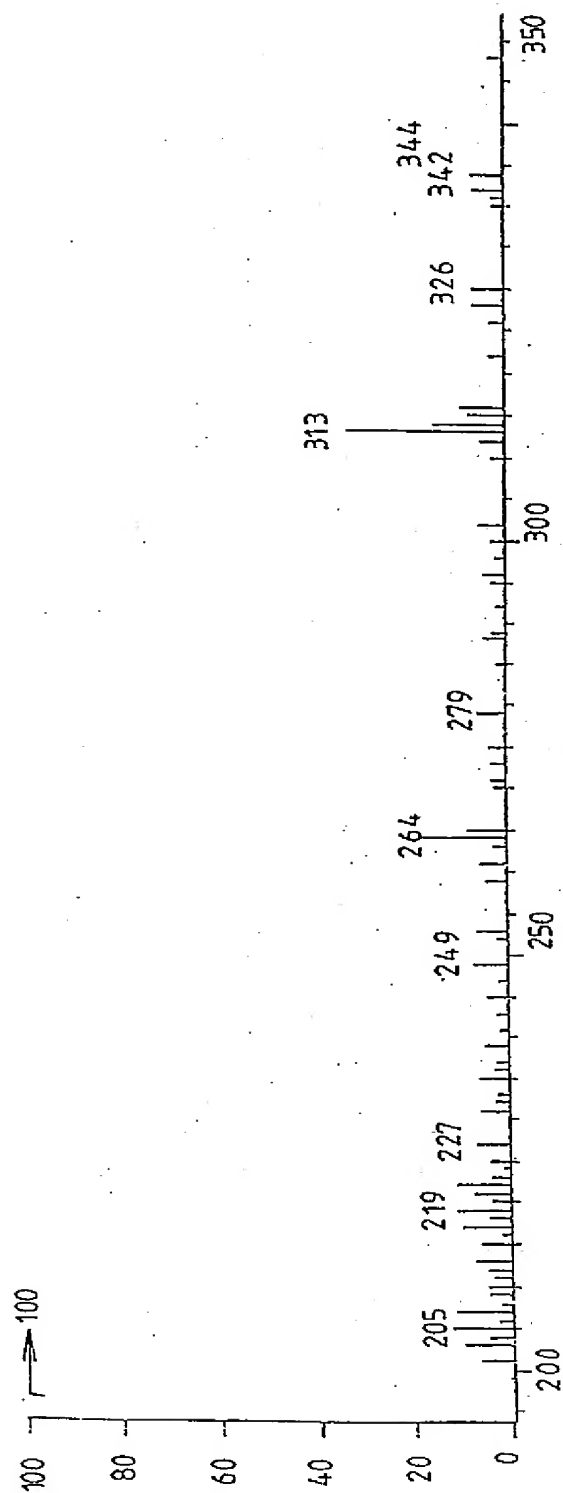
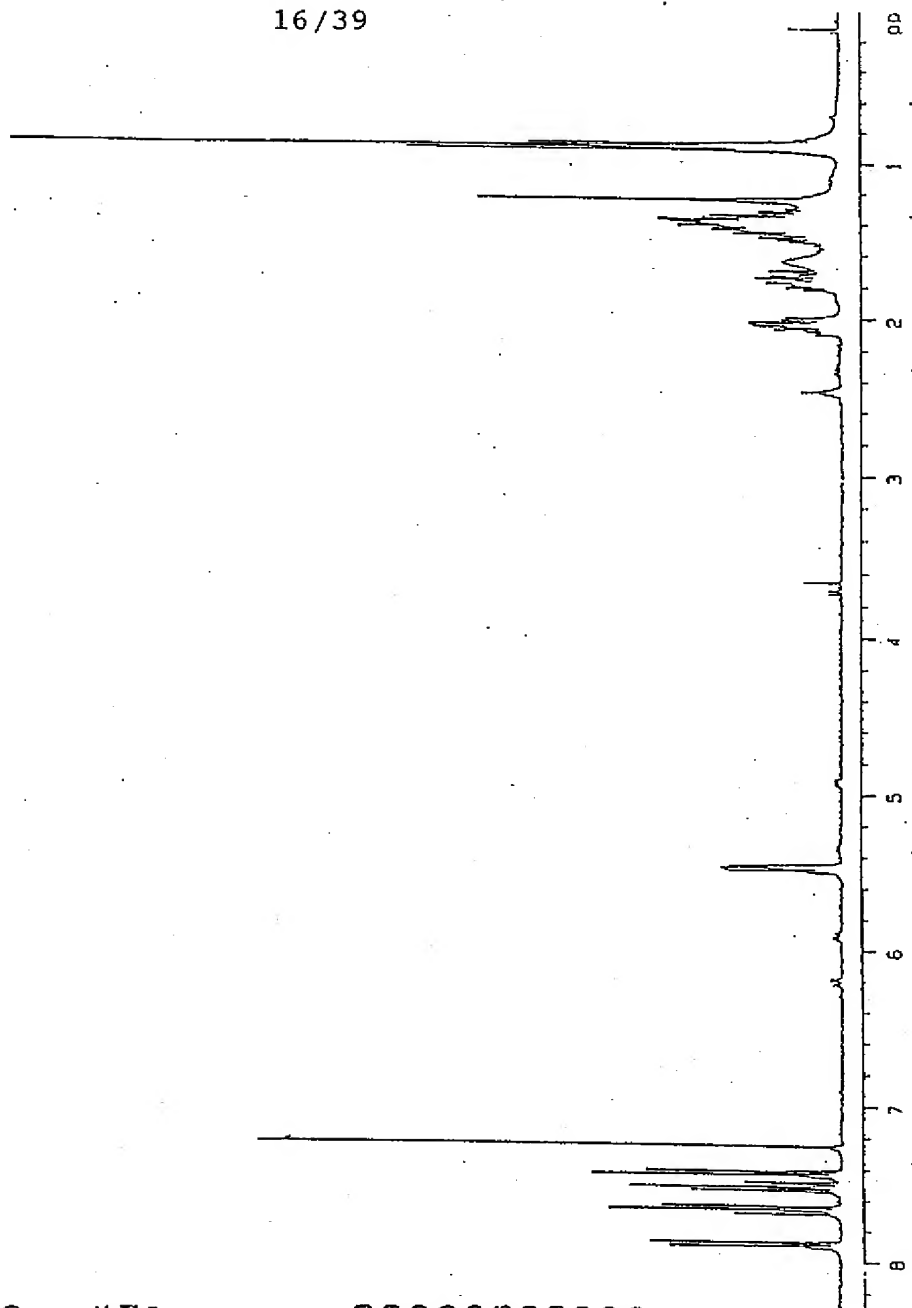


FIG 15B

16 / 39



exp2 pulse sequence. stdh

SAMPLE		DEC. & VT	
date	May 15 93	dn	H1
solvent	CDCl3	dof	0
file	/home/vnmr	dm	non
rf/vnmrswa/data/Da		dmm	200
unter_JH_2D_2		dmf	30
ACQUISITION		dpwr	26.0
sfreq	299.949	temp	PROCESSING
th	H1		
bt	3.745	wtfile	ft
np	18944	proc	not used
em	2528.9	fn	f
fb	1400	math	
bs	16		
tpwr	55	werr	
pw	26.0	wexp	
di	0	wbs	
tof	149.3	wnt	
nt	32		DISPLAY
ct	32	ap	-38.0
block	n	wp	2528.9
gain	30	vs	200
		sc	0
ll	n	wc	330
ln	n	hzrm	7.68
dp	y	ls	1936.10
hs	nn	rfl	38.8
		rfp	0
		th	20
		ins	1.000
		nm	cdc
			ph

FIG 18

17/39

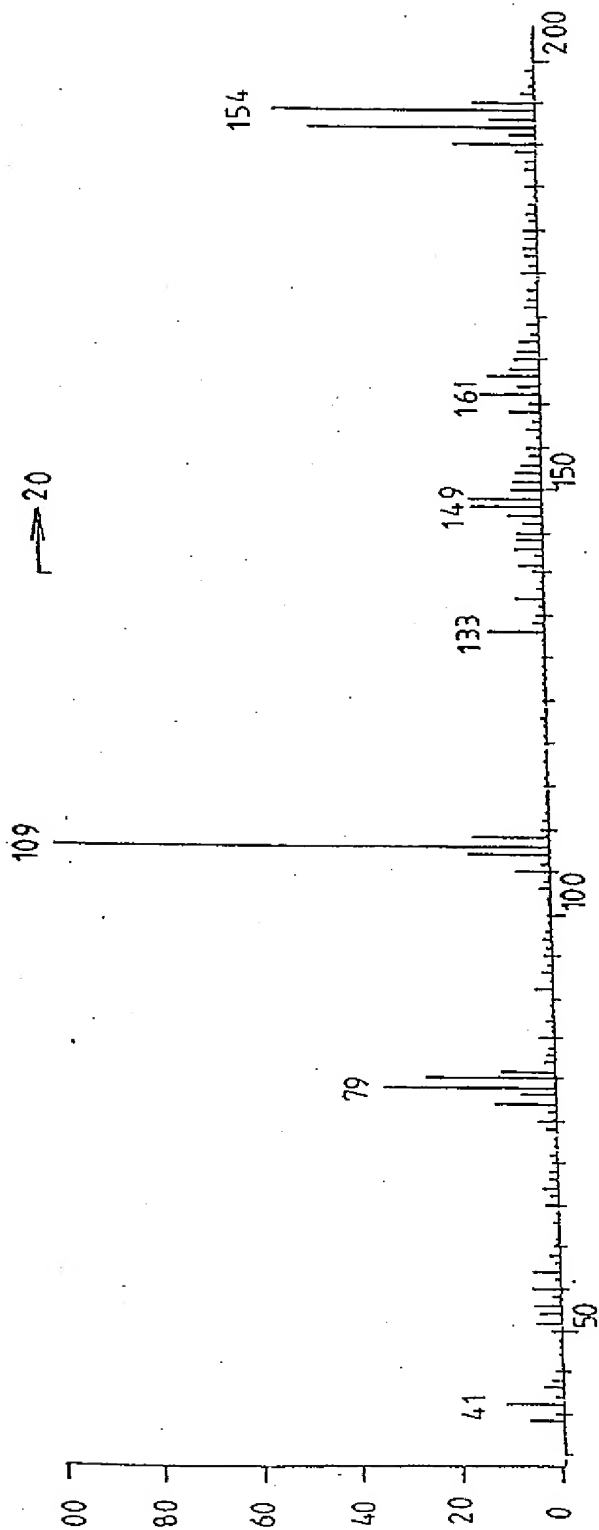


FIG 17A

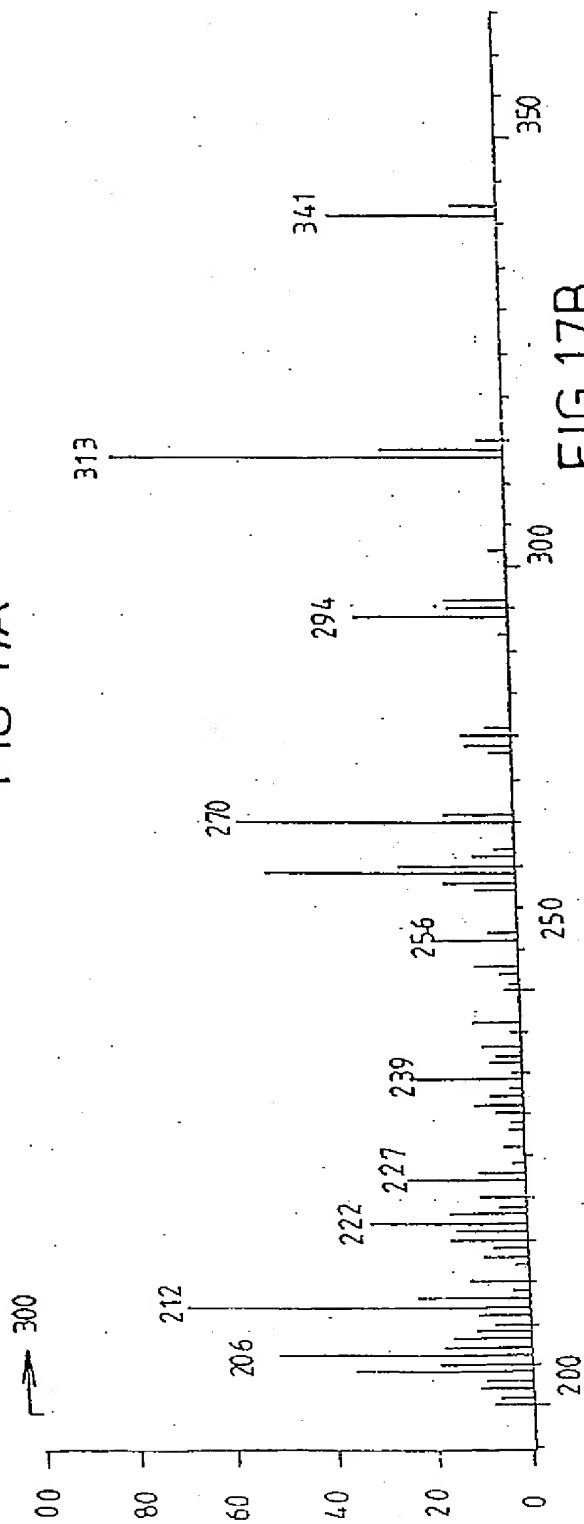


FIG 17B

18/39

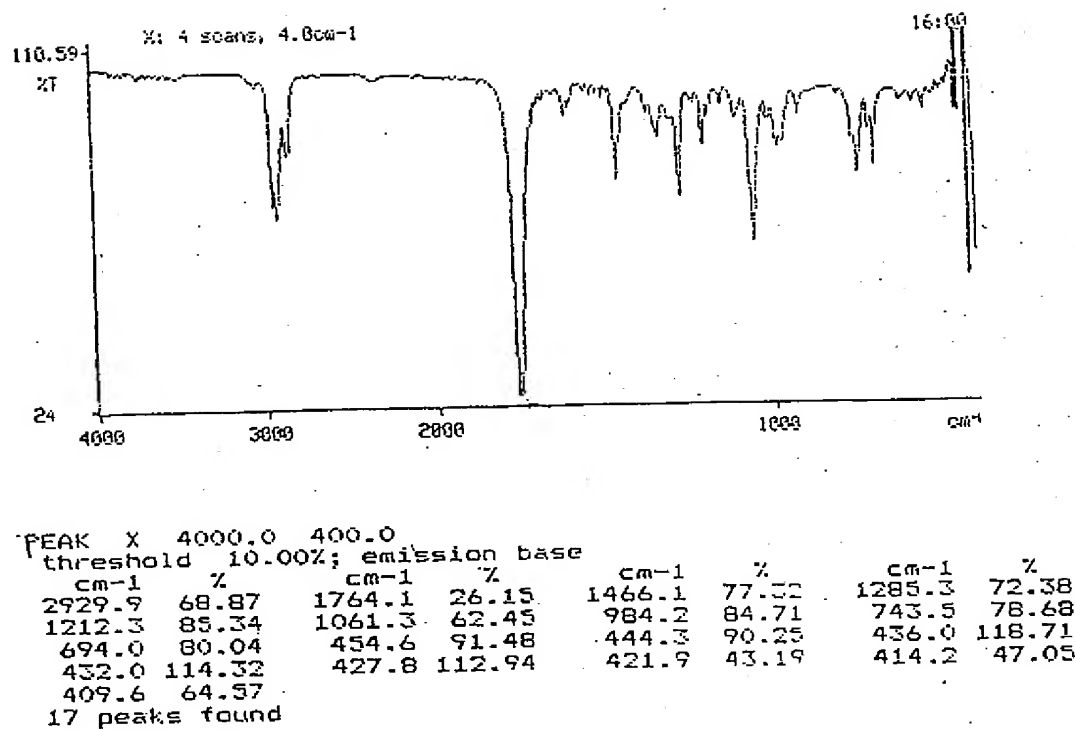


FIG. 18

19/39

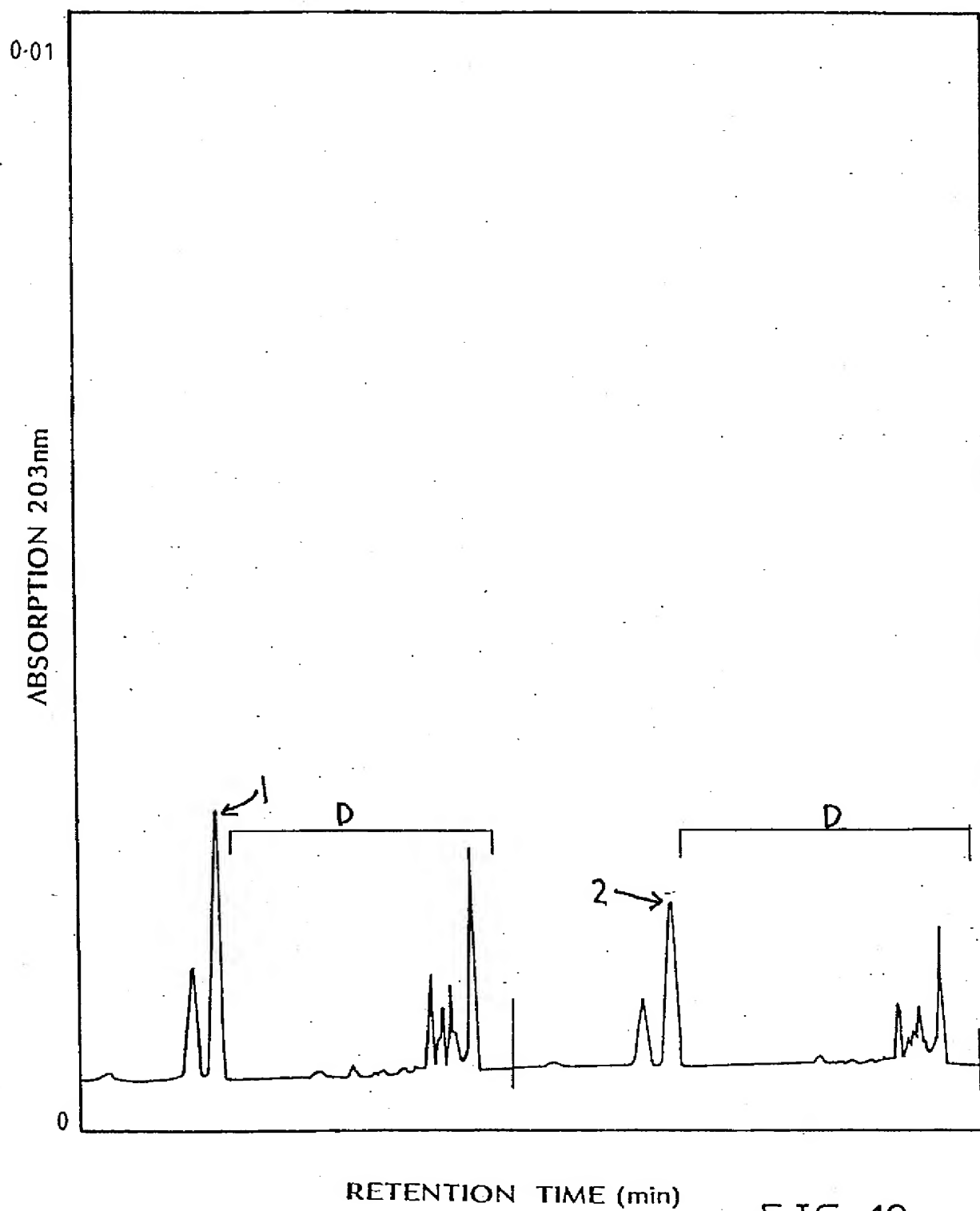
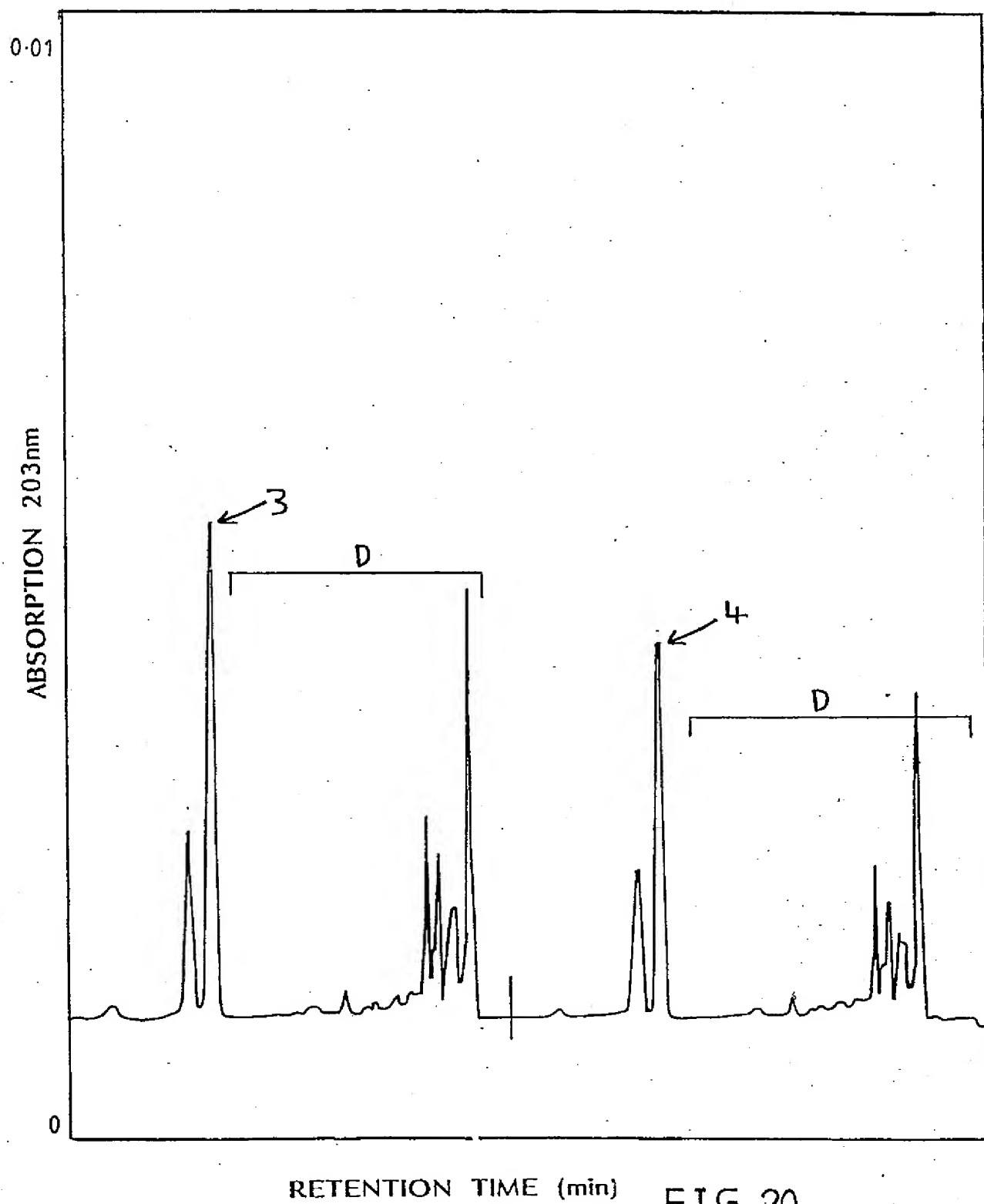
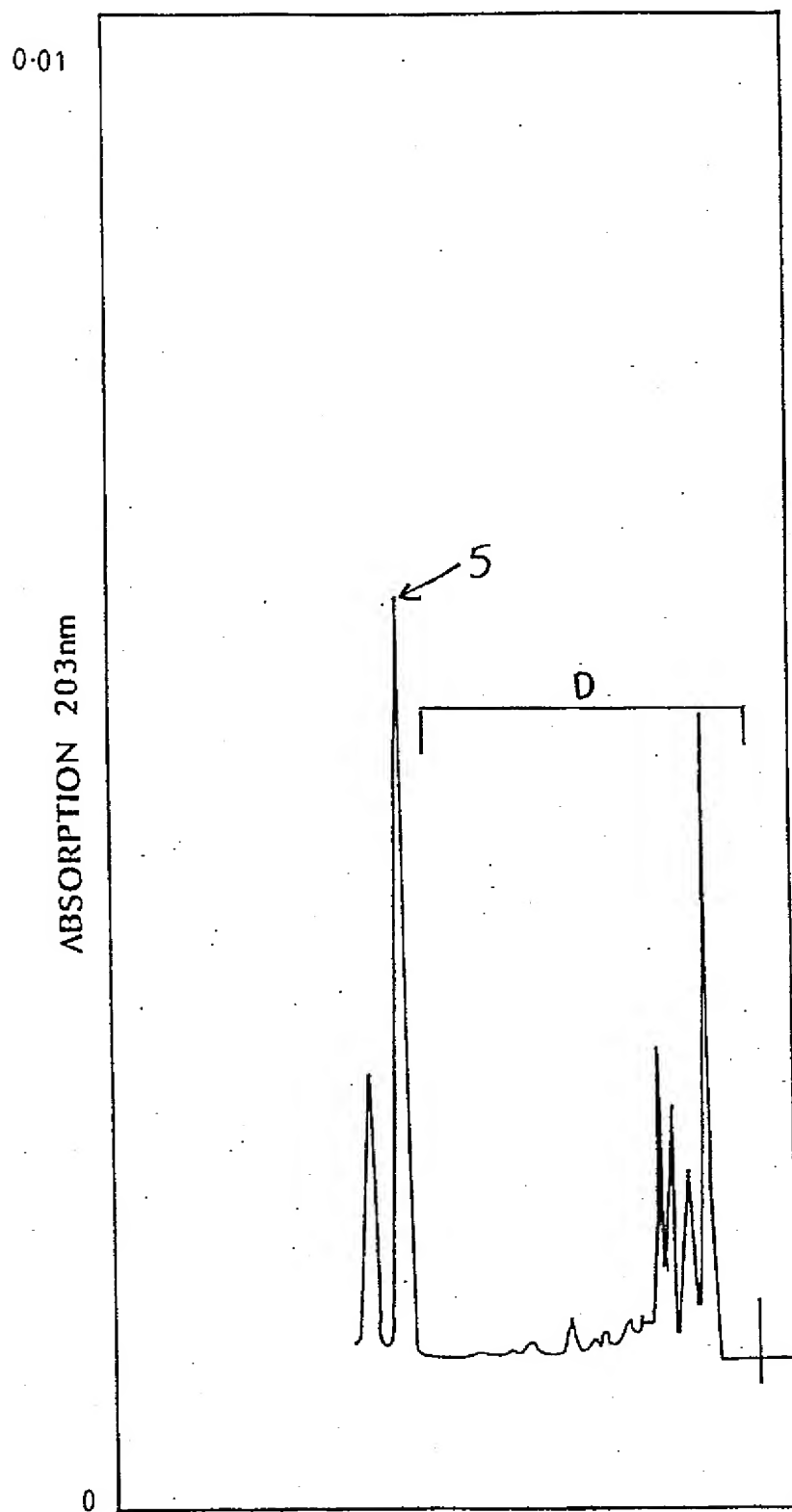


FIG. 19

20/39



21/39



RETENTION TIME (min) FIG. 21

22/39

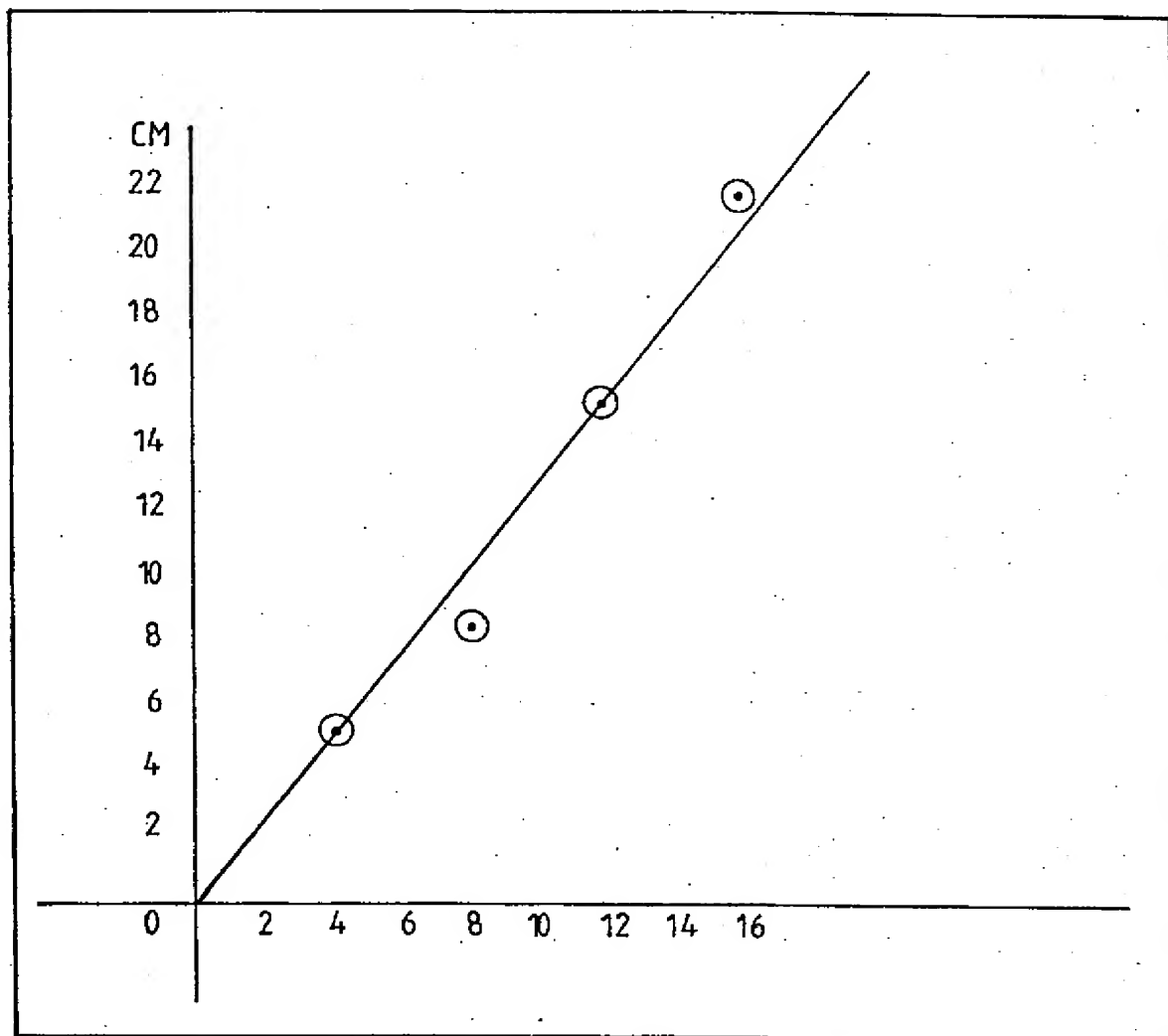


FIG. 22

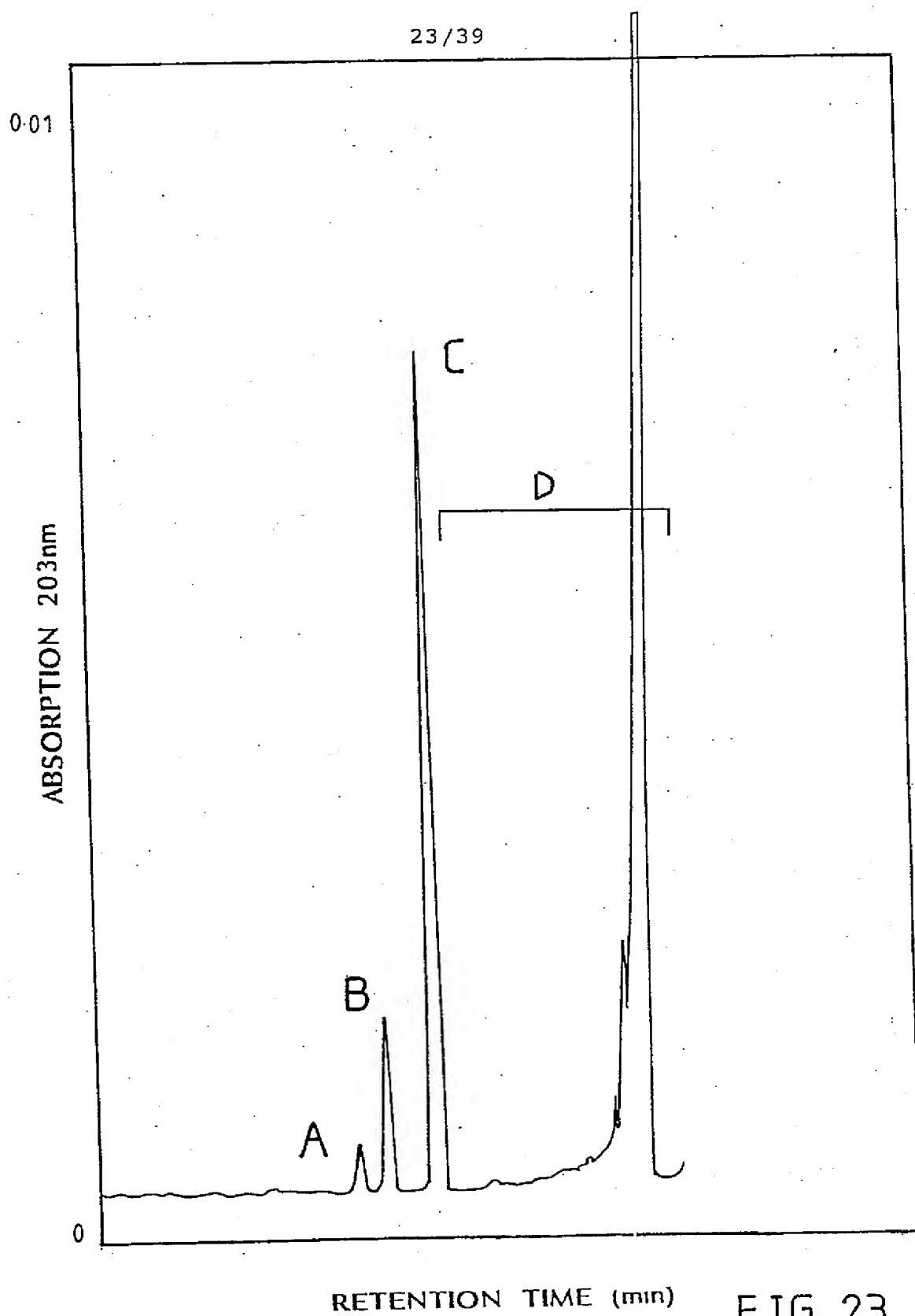
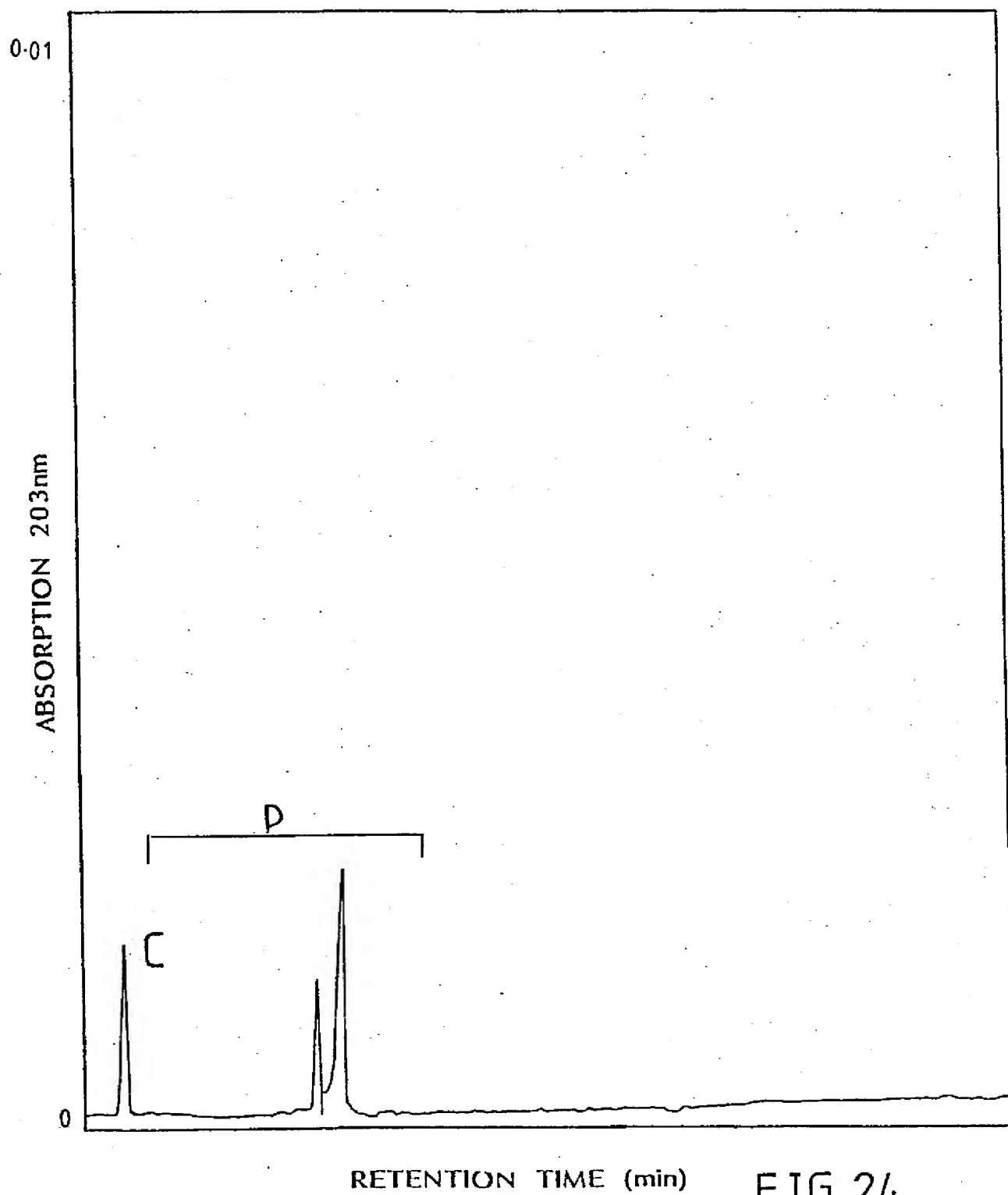


FIG. 23

24/39



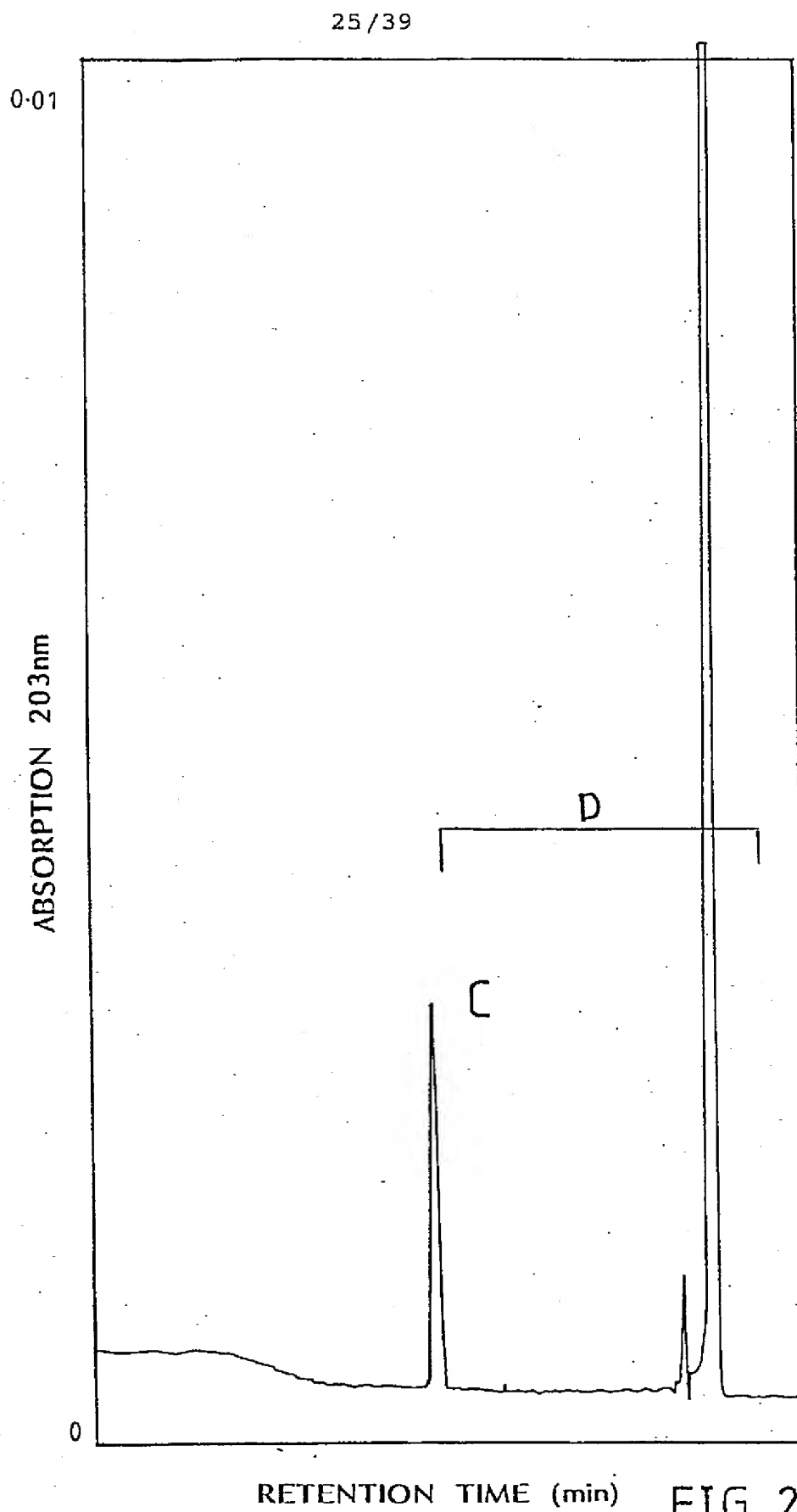


FIG. 25

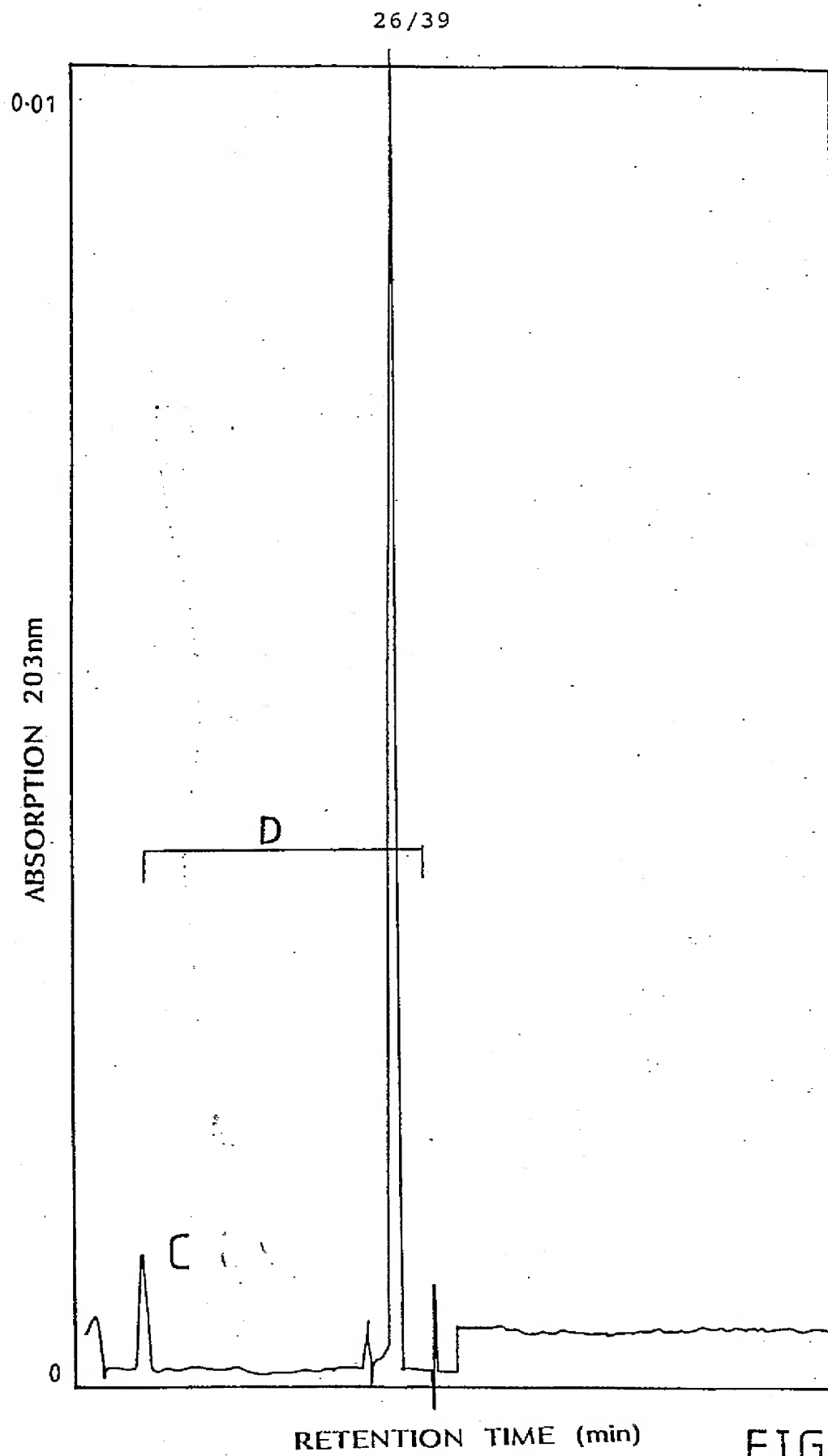


FIG. 26

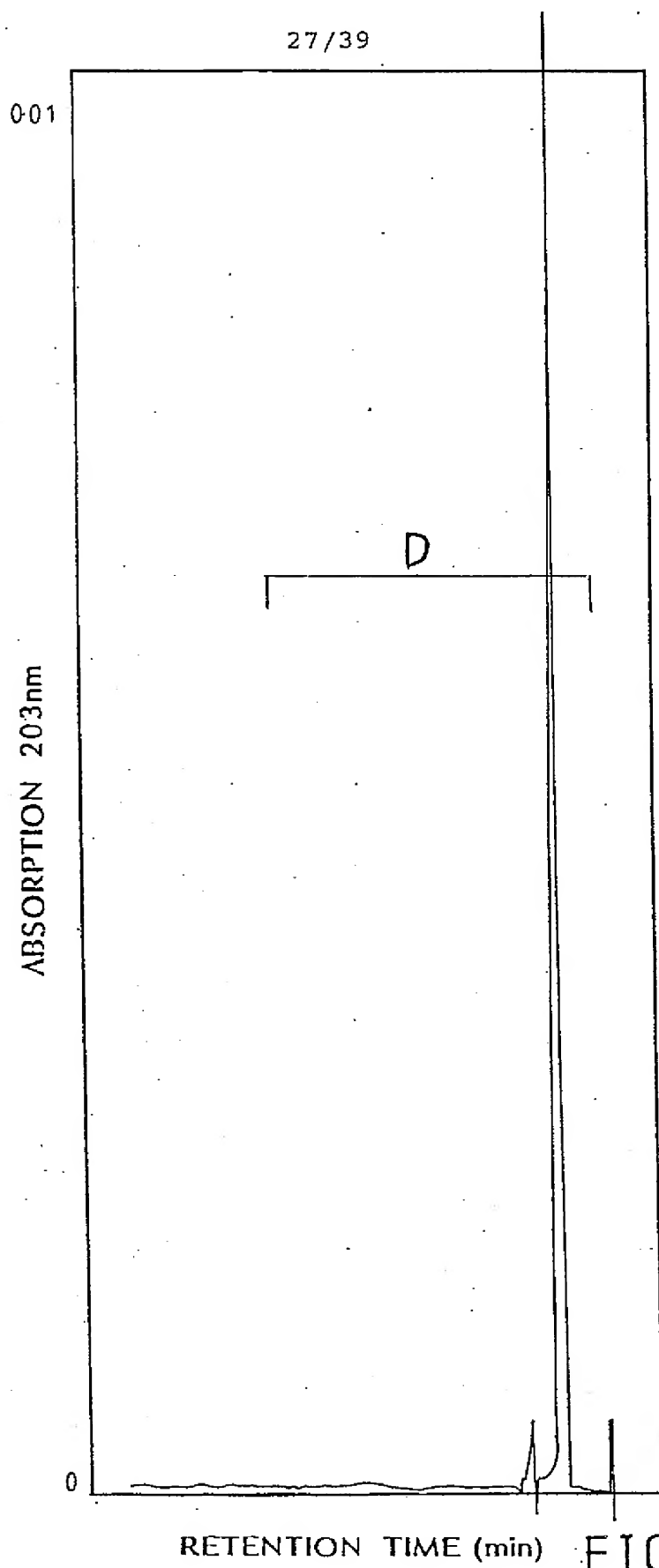
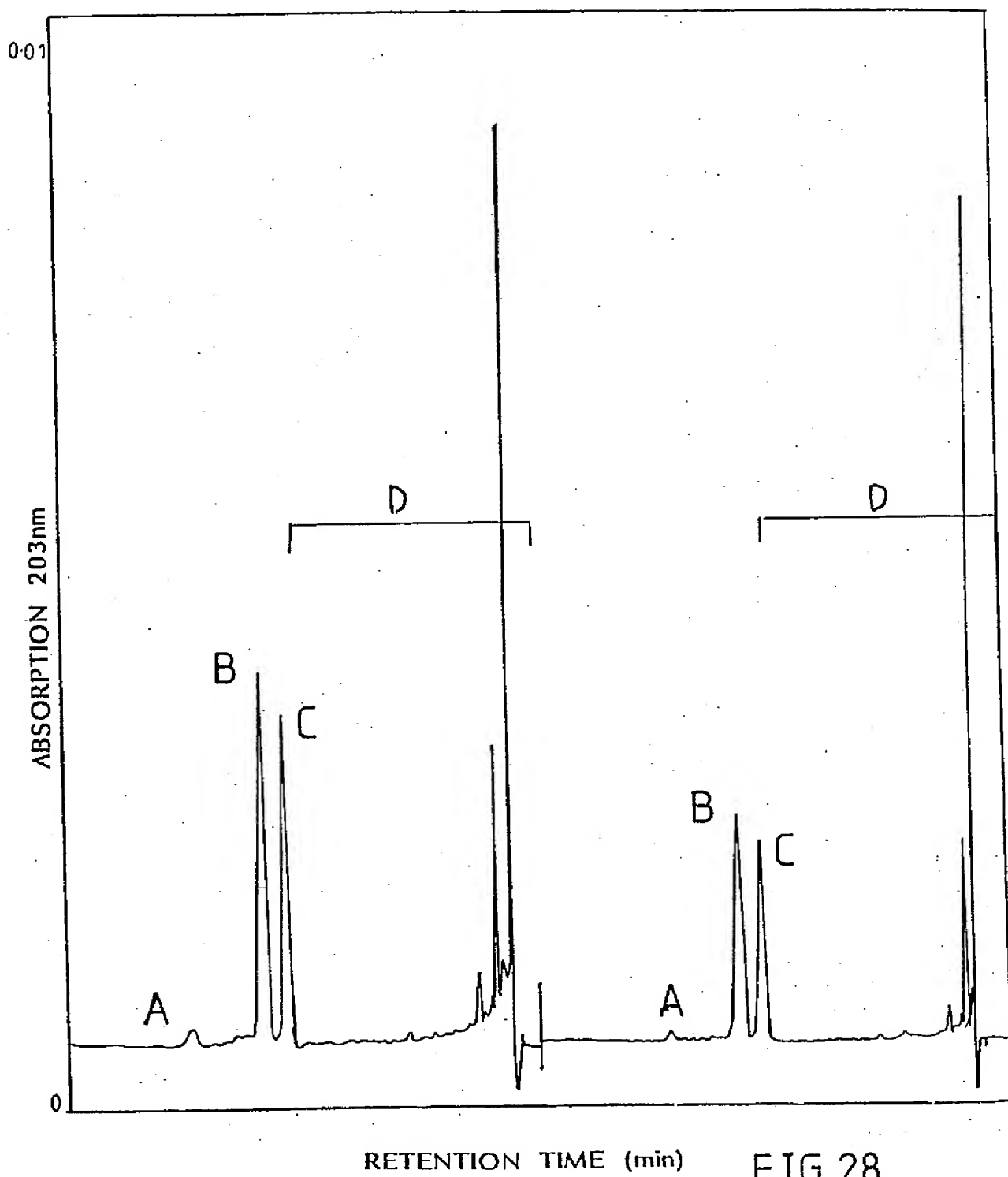


FIG. 27

28/39



29/39

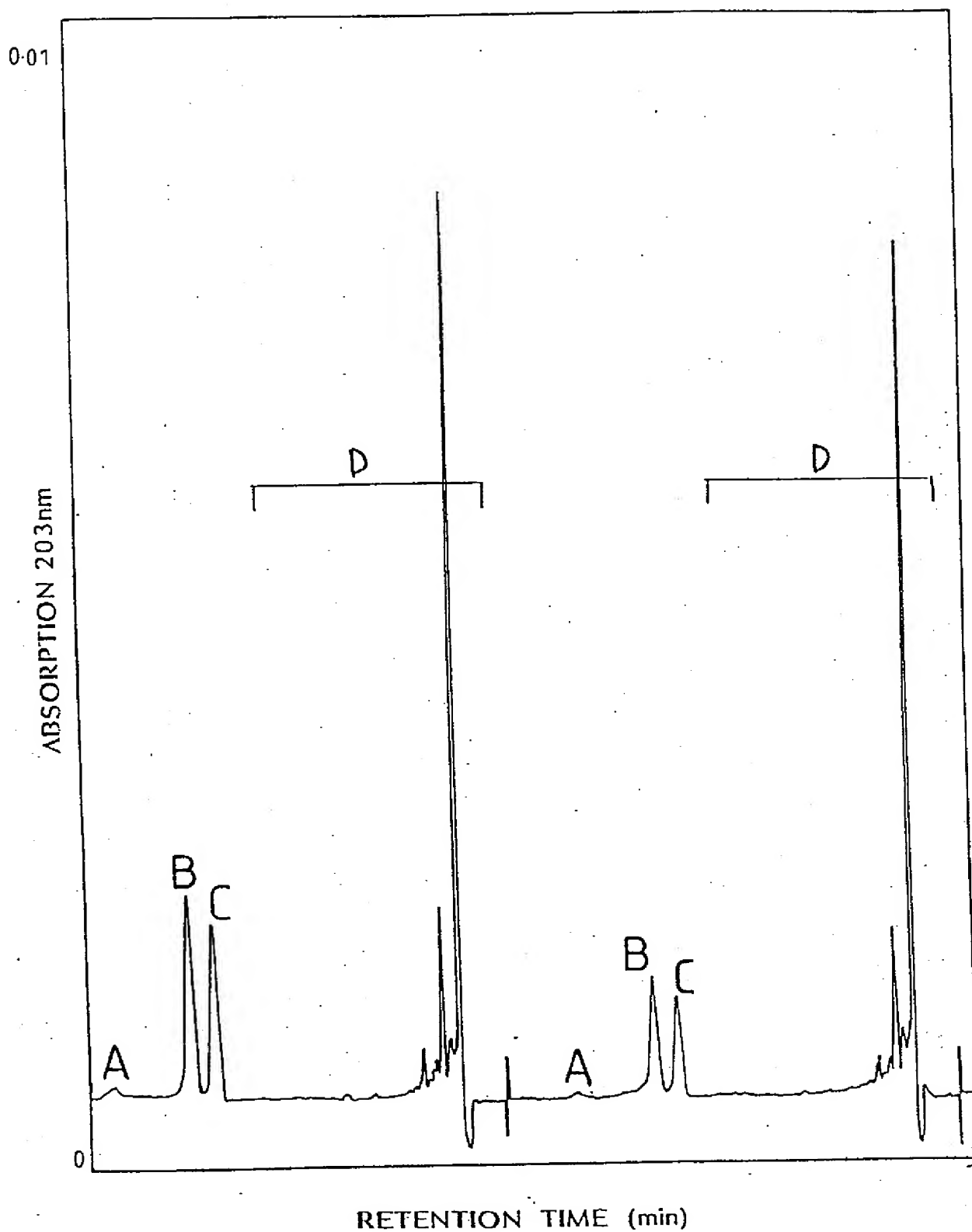


FIG. 29

30/39

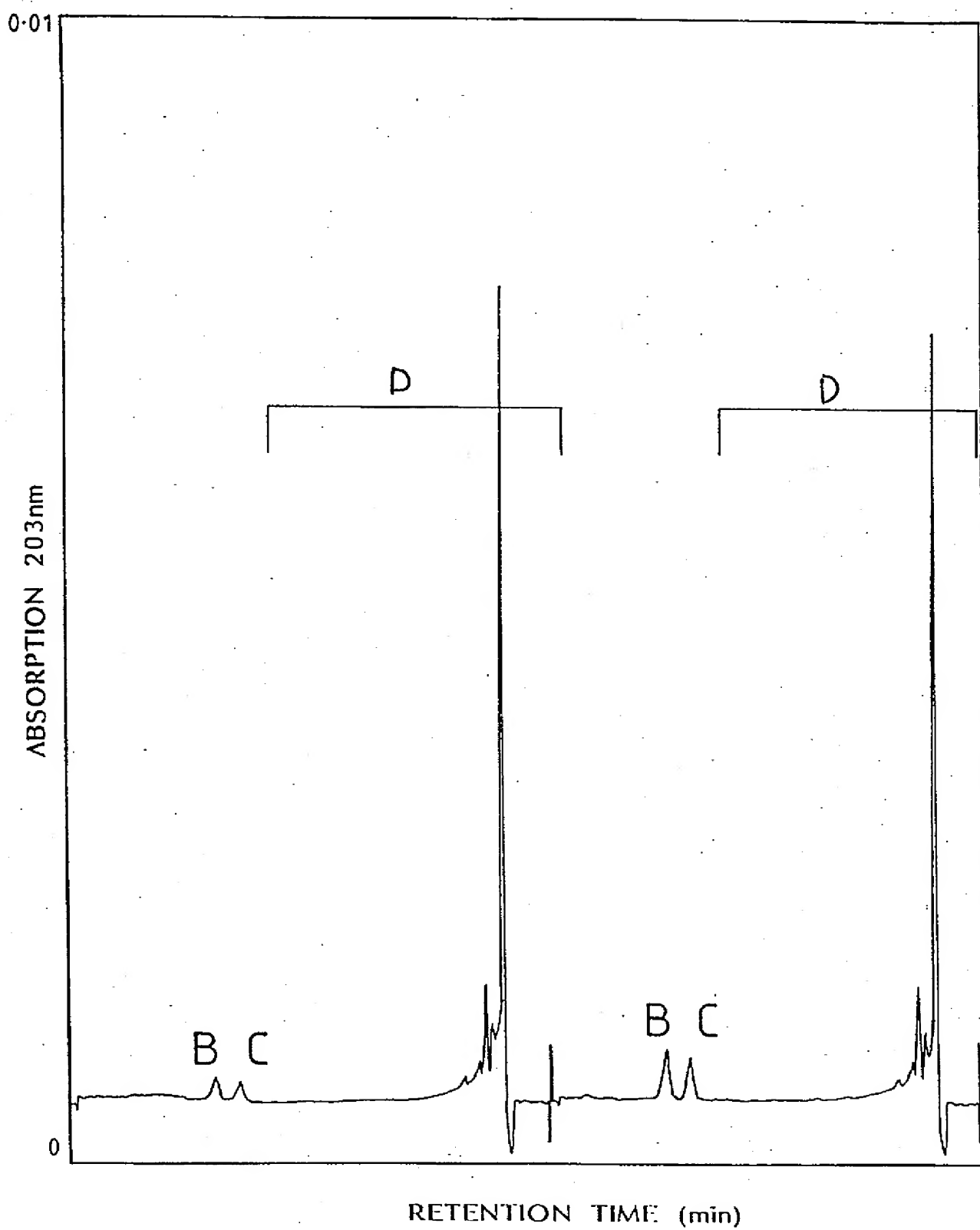


FIG. 30

31/39

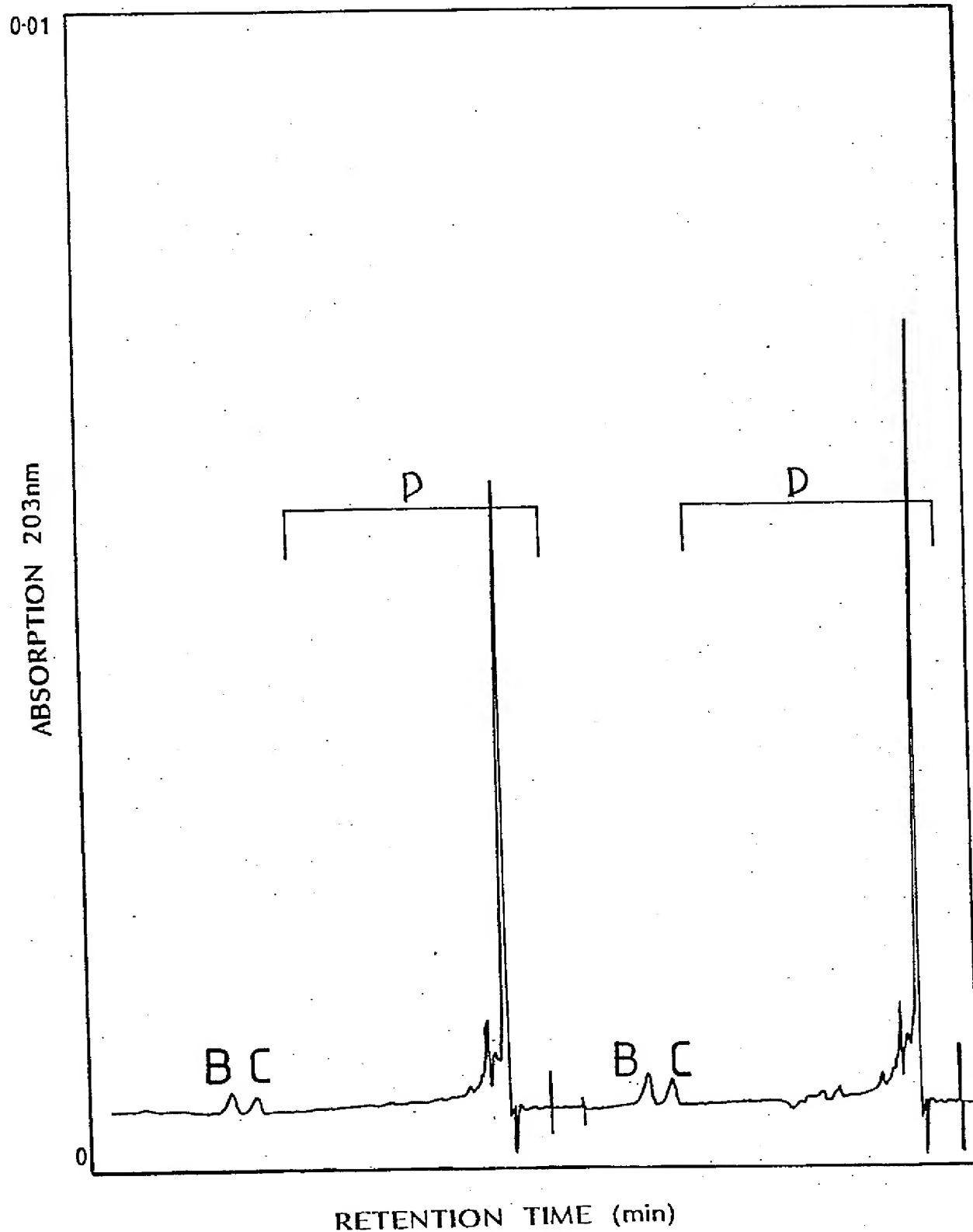


FIG. 31

32/39

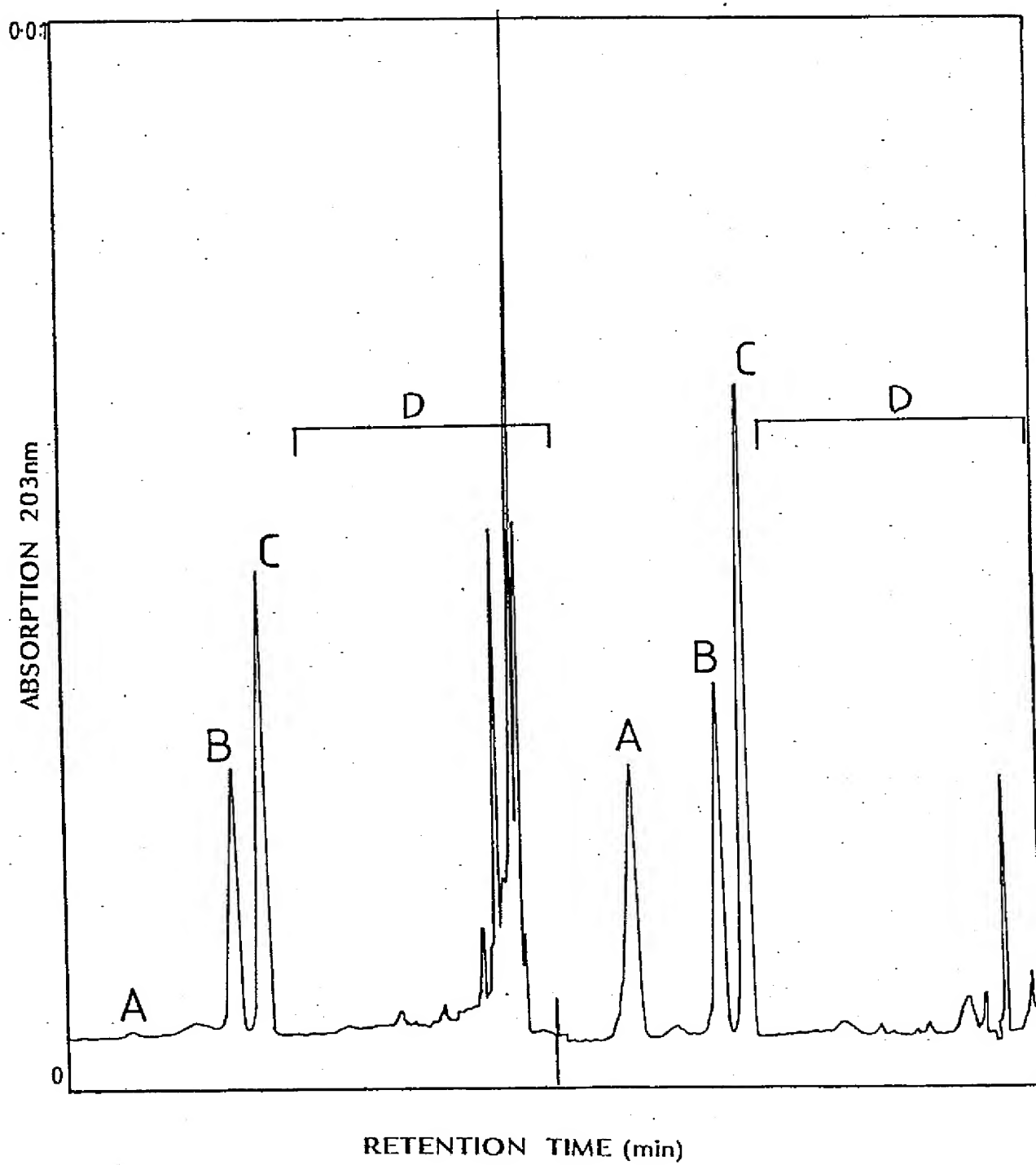


FIG. 32

33/39

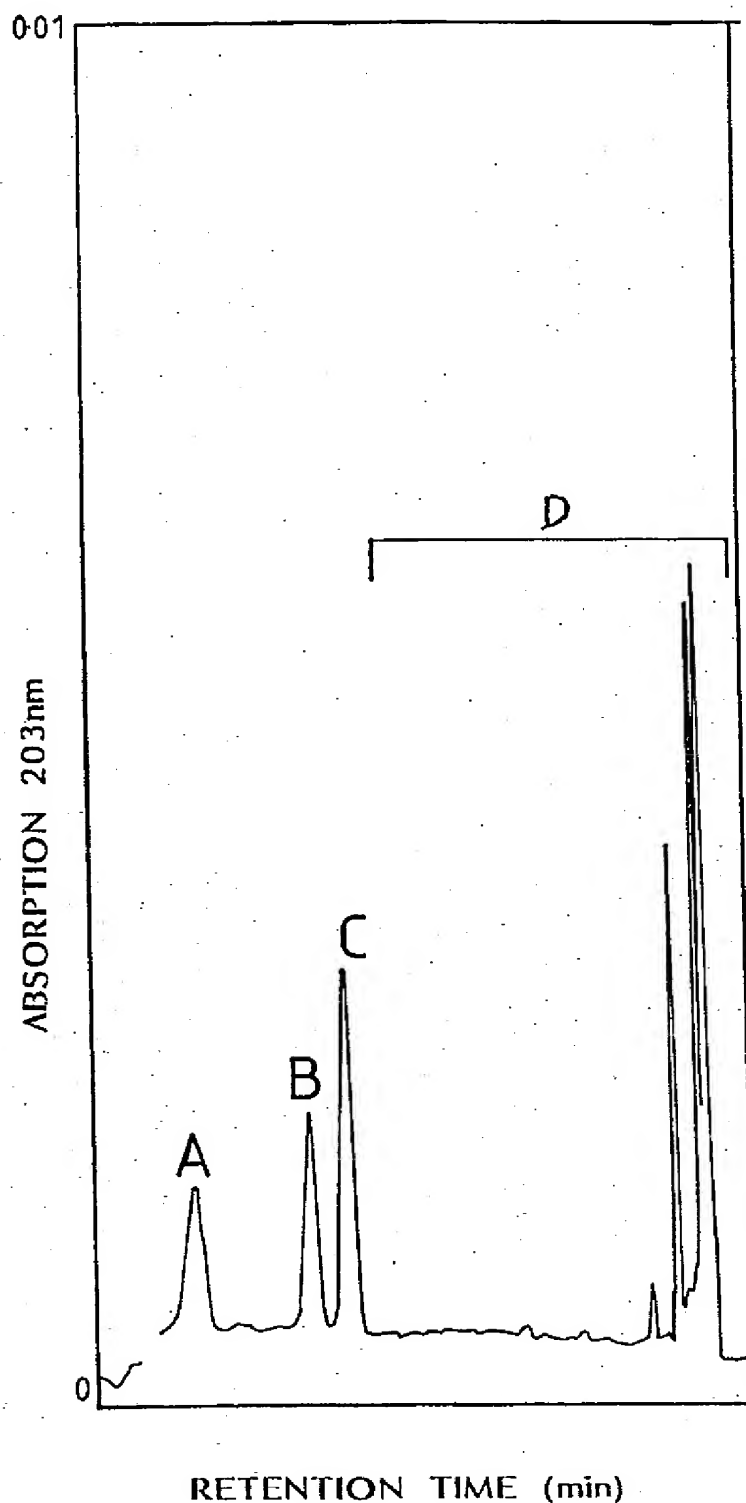


FIG. 33

34/39

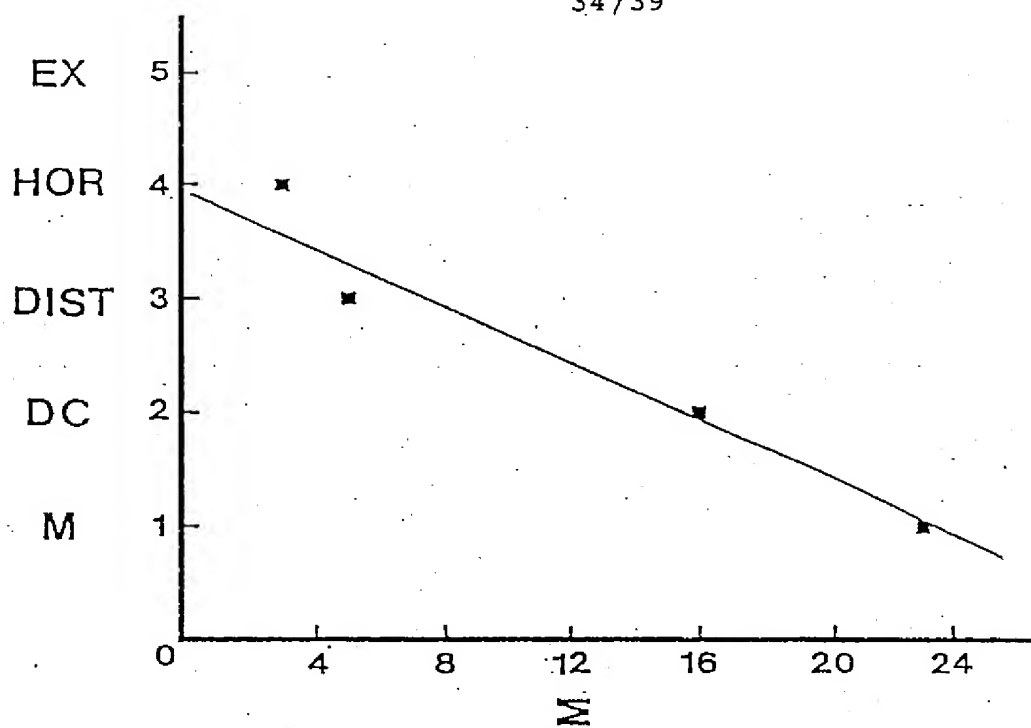


FIG 34

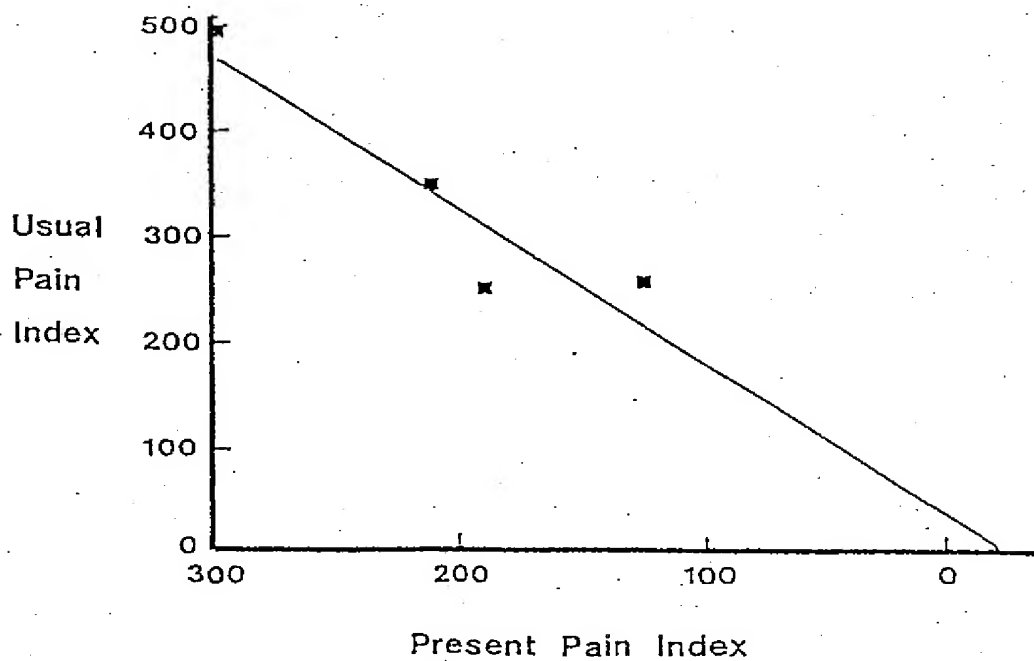


FIG 35

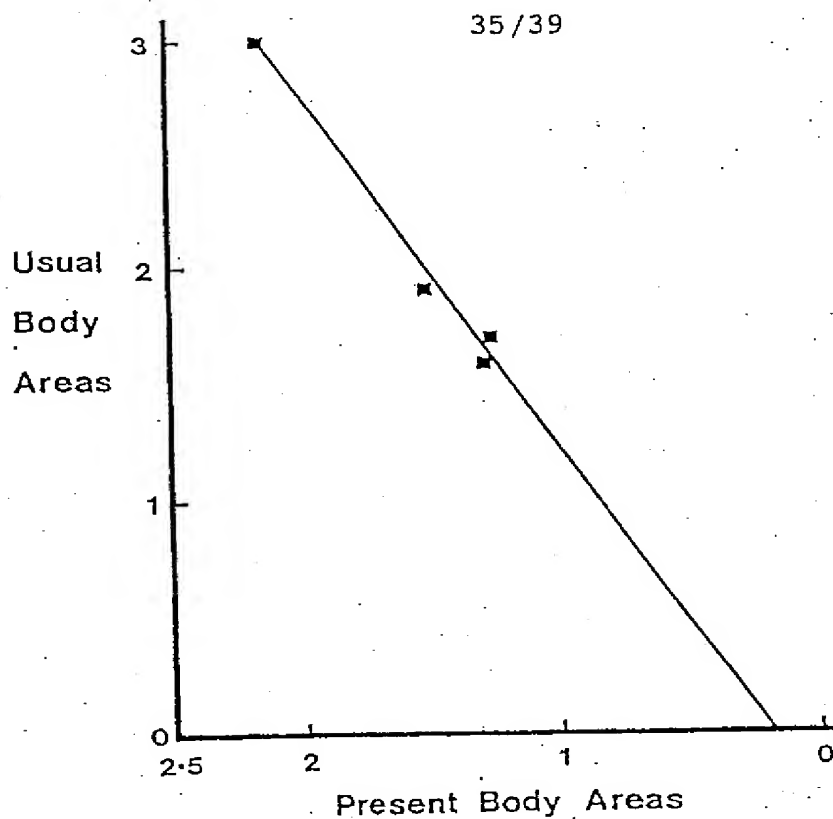


FIG 36

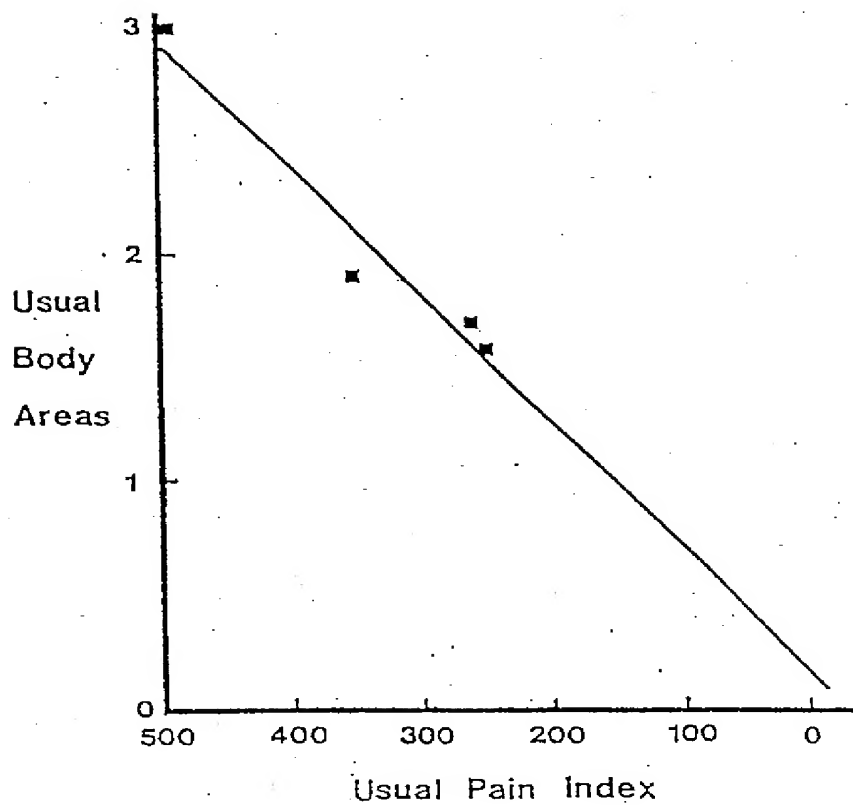


FIG 37

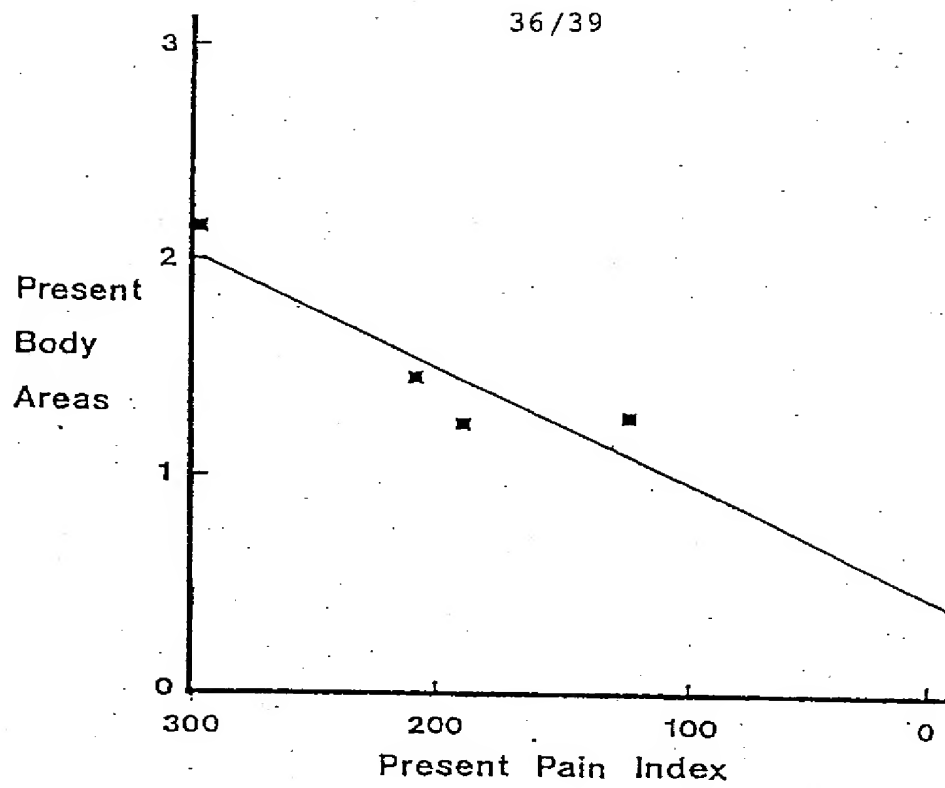


FIG 38

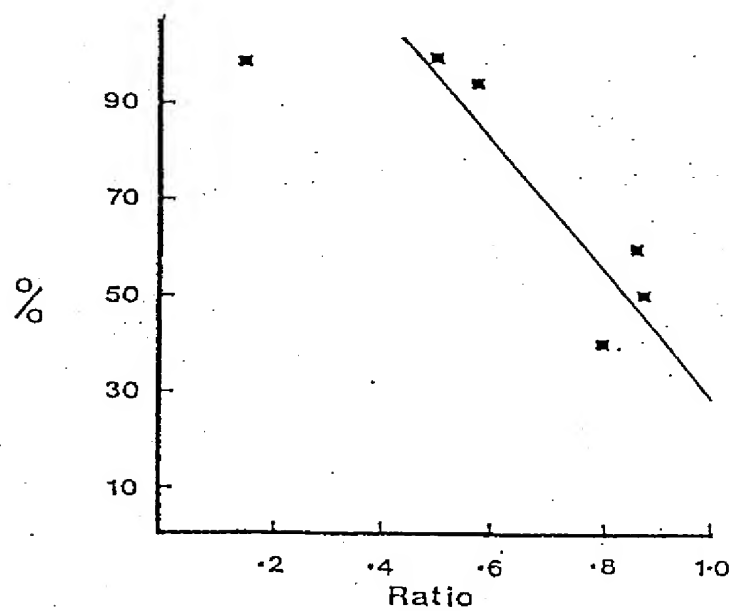


FIG 39

37/39

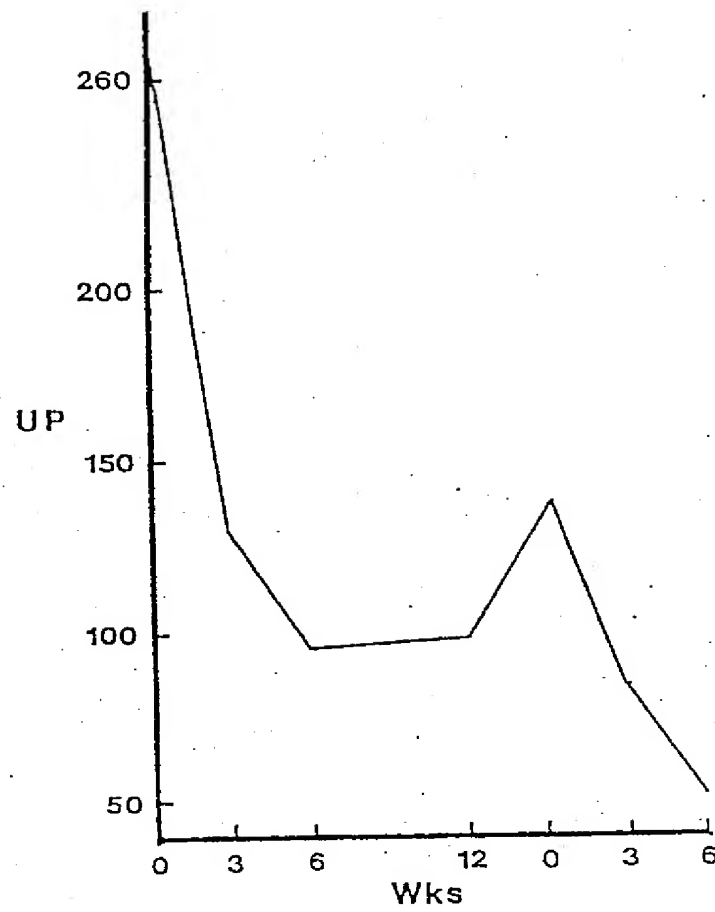


FIG 40

38/39

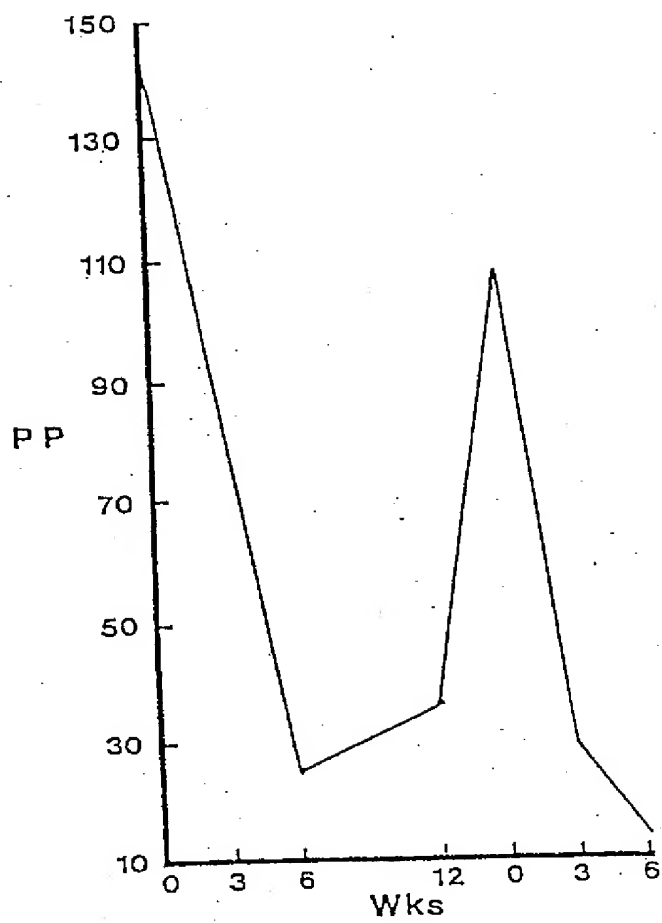


FIG 41

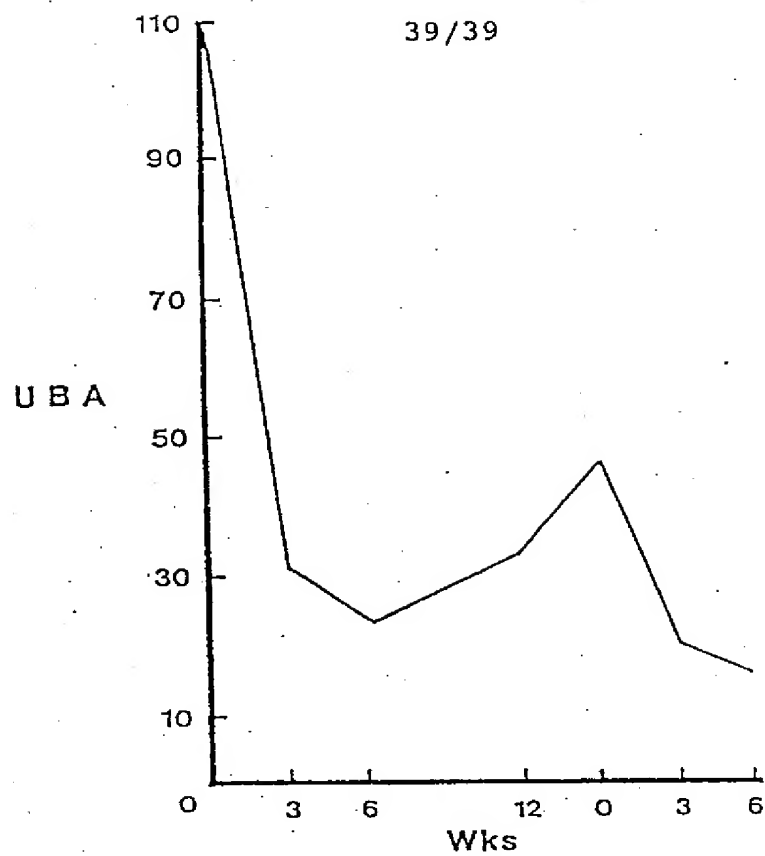


FIG 42

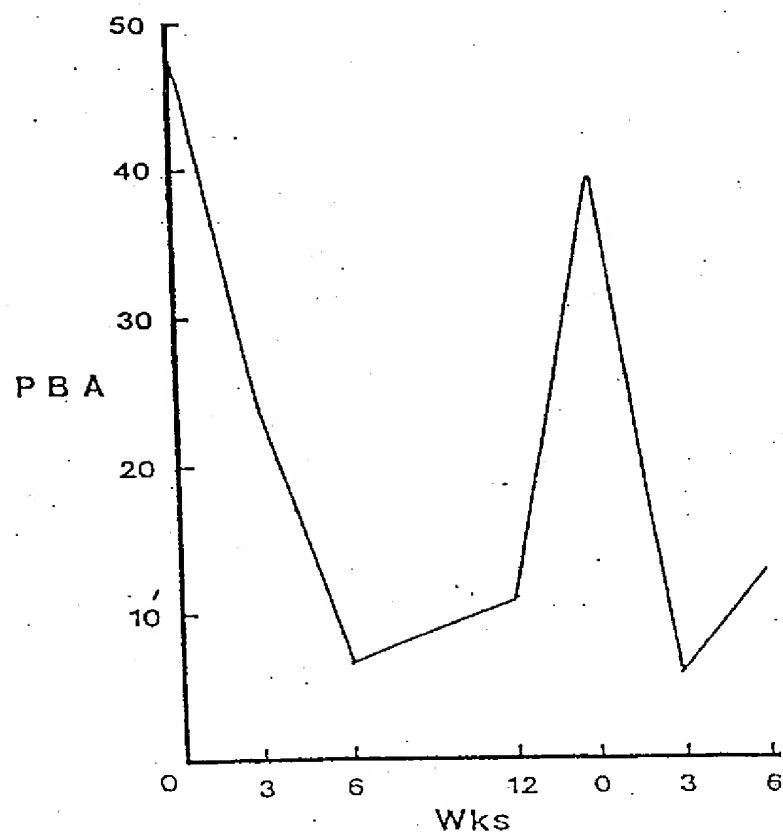



FIG 43

A. CLASSIFICATION OF SUBJECT MATTER Int. Cl. ⁵ A61K 35/78, 31/365, 31/34, 31/19, 31/12, 31/16, 31/40 According to International Patent Classification (IPC) or to both national classification and IPC				
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC A61K 35/78, 31/365, 31/34, 31/19, 31/12, 31/16, 31/40 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched AU:IPC as above Electronic data base consulted during the international search (name of data base, and where practicable, search terms used) ORBIT: antiinflamm or inflamm (1975-92) CASM: butylphthalide or sedanenolide				
C. DOCUMENTS CONSIDERED TO BE RELEVANT				
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.		
X	EP,A,516588 (CIBA GEIGY AG) 2 December 1992 (02.12.92) the whole document	1,3-7		
X	EP,A,365210 (KYOWA HAKKO KOGYO CO., LTD) 25 April 1990 (25.04.90) the whole document	1,3-7		
X	EP,A,147778 (TROPONWERKE GmbH & CO KG) 10 July 1985 (10.07.85) the whole document	1,3-7		
X	EP,A,488965 (SIGMA-TAU INDUSTRIE FARMACEUTIQUE RIUNITE SPA) 3 June 1992 (03.06.92) the whole document	1,3-7		
X	US,A,4868153 (ALLISON, A.C. et al) 19 September 1989 (19.09.89) the whole document	1,3,7		
<div style="display: flex; justify-content: space-between; align-items: flex-start;"> <div style="width: 45%;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. </div> <div style="width: 45%;"> <input checked="" type="checkbox"/> See patent family annex. </div> </div>				
<table style="width: 100%; border: none;"> <tr> <td style="width: 50%; vertical-align: top;"> <p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </td> <td style="width: 50%; vertical-align: top;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p> </td> </tr> </table>			<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>
<p>* Special categories of cited documents :</p> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art</p> <p>"&" document member of the same patent family</p>			
Date of the actual completion of the international search 25 August 1994 (25.08.94)		Date of mailing of the international search report <i>1 Sept 1994 (01.09.94)</i>		
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No. 06 2853929		Authorized officer  John Hanson Telephone No. (06) 2832262		

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate of the relevant passages	Relevant to Claim No.
	WO,A,91/06863 (E.I. DU PONT DE NEMOURS AND COMPANY) 16 May 1991 (16.05.91)	
X	The whole document	1,3-7
	WO,A,87/01698 (THE UPJOHN COMPANY) 26 March 1987 (26.03.87)	
X	page 8 line 36 - page 9 line 12 and claim 1	1,3-7
	Derwent Abstract Accession No. 86-187794, class B04, JP,A,61-122221 (OSAKA YAKUHI KEN K) 10 June 1986 (10.06.86)	
X	Abstract	1-8,18,20
Y	<u>Journal of Agricultural and Food Chemistry</u>	15-17,21
	Volume 40, No.6, issued 1992 (Nitz, S. et al) "3-butyl-5,6-dihydro-4H-isobenzofuran-1-one, a sensorial active phthalide in parsley roots	
Y	pages 1038-1040	1-8,15-18,20,21
	<u>The Chemistry of Essential Oils and Artificial Perfumes</u> (Parry, E.J.), 2 nd Edition, published by Scott Greenwood & Son, 1908	
X	Pages 345-347 and 350-353	8-10,14,16,18,19
Y	<u>Journal of Association of Official Analytical Chemists, Inc.</u> Volume 61, No.5, issued 1978 (Lund, E.D.) "Thin layer and high pressure liquid chromatographic analysis of celery seed oil"	1-7,11-13,15,17,20-22
X	Pages 1083-1088	8-10,14,16,19
Y	<u>Journal of Organic Chemistry</u> Volume 42, No.13, issued 1977 (Bjeldanes, L.F. et al) "Phthalide components of celery essential oil"	1-7,11-13,15,17,18,20,22
X	Pages 2333-2335	8-10,14,16,19,22
	<u>Journal of Food Science</u> Volume 43, No.1 issued 1978 (Bjeldanes, L.F. et al)	
X	"Sedative activity of celery oil constituents"	1-7,11-13,15,17,20,21
Y	Page 143	8-10,14,16,18,19,22
	Derwent Abstract Accession No. 83-818134, Class B04, JP,A,58-172321 (DAINIPPON PHARM KK) 11 October 1983 (11.10.83)	
X	Abstract	8,10,14,15,17,18
Y	FR,A,2611502 (LAB NATURA MEDICA) 9 September 1988 (09.09.88)	9,11-13
X	The whole document	8,11,12,15,16,18
Y		17
X	FR,A,2334368 (UNILEVER NV) 12 August 1977 (12.08.77)	8,10,11,15,16,18
Y	The whole document	9,12,13,14,17

INTERNATIONAL SEARCH REPORT

International application No.

PCT/AU 94/00342

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member
EP 516588	AU,A,17053/92 CA,A,2069857 CN,A,1067052 FI,A,922459 MX,A,9202544 NO,A,922133 PL,A,294701 JP,A,5163240
EP 365210	JP,A,2196767
EP 147778	DE,A,3347658 JP,A,60158149 PH,A,21602 US,A,4628062 ZA,A,8410112
EP 488965	IT,A,1242020
US 4868153	AU,A,82540/87 DK,A,6587/87 EP,A,281713 FI,A,875502 HK,A,1258/93 IL,A,84833 JP,A,63188672 NO,A,875240 NZ,A,222922 PH,A,23819 PT,A,86343 US,A,4753935 ZA,A,8709414
WO 91/06863	AU,A,66143/90 EP,A,500586 NZ,A,235914 US,A,5102883 ZA,A,9008745
WO 87/01698	EP,A,246245 US,A,4939169
FR 2334368	IT,A,1091073
END OF ANNEX	